Enclosures - letter from Dr. Gerald McEwen, CTFA, on June 8, 2005 in response to request for additional information/public comments on Toxicological Program (70 Federal Register 23877): Butylparaben

Final Report. Charles River Discovery And Development Services Argus Division. Protocol 1203-006. Oral (Diet) Reproduction Toxicity Study Of Butylparaben In Male Rats. Final Report Date: March 17, 2005. 257 Pages.

Report Amendment 1. Charles River Discovery And Development Services Argus Division. Protocol 1203-006. Oral (Diet) Reproduction Toxicity Study Of Butylparaben In Male Rats. 15 April 2005. 4 pages.

Methylparaben and Butylparaben: In Vitro Dermal Penetration and Metabolism in Rat and Human Skin. E.I. du Pont de Nemours and Company HaskellSM Laboratory for Health and Environmental Sciences. Laboratory Project ID: DuPont-13966. November 22, 2004. 175 pages.

Butylparaben: In Vitro Dermal Penetration and Metabolism Using Full Thickness Human Skin. E.I. du Pont de Nemours and Company HaskellSM Laboratory for Health and Environmental Sciences. Laboratory Project ID: DuPont-15565. November 17, 2004. 54 pages.

FINAL REPORT

CHARLES RIVER
DISCOVERY AND DEVELOPMENT SERVICES
ARGUS DIVISION

PROTOCOL 1203-006

ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

FINAL REPORT DATE: MARCH 17, 2005

TITLE: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

CR-DDS ARGUS DIVISION PROTOCOL NUMBER: 1203-006

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1. SUMMARY AND CONCLUSION

The purpose of this study was to test for toxic effects/disturbances resulting from oral (diet) exposure to butylparaben of Crl:(WI) BR male rats on spermatogenesis.

1.1. Study Design[a]

Sixty-four male rats were assigned to four exposure groups, 16 male rats per group. Prepared diets containing the test article, butylparaben, at constant concentrations of 0, 100, 1000 and 10000 ppm were available *ad libitum* to the rats for a minimum of 56 days. All rats were 22 days of age when first exposed to test diets, with exposure continuing until the day of sacrifice. Viabilities, clinical observations, body weights and feed consumption values were recorded. Beginning at the start of week 3 of the exposure period, blood samples were collected every other week from each male rat assigned to study and analyzed at the Testing Facility for LH (luteinizing hormone), FSH (follicle-stimulating hormone) and testosterone.

On the day of sacrifice, all surviving F1 generation male rats were sacrificed, and blood samples were collected for possible future analysis for butylparaben and para hydroxy benzoic acid levels. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Reproductive organs from all rats, as well as the liver, adrenal glands, thyroid and pituitary gland (six per group), were weighed and retained for possible histological evaluation. Sperm evaluations were conducted to determine sperm concentration, motility and morphology. The left testis from each rat was collected for evaluation of Daily Sperm Production (DSP) determinations (i.e. testicular spermatid concentration). The liver, adrenal, thyroid and pituitary glands from ten male rats per exposure group were quick-frozen in liquid nitrogen and retained for possible hormone measurements. Histological examination was performed on the reproductive organs from all rats assigned to the control and high test article concentration groups. Additionally, the liver, adrenals, thyroid and pituitary glands from six rats in the control and high test article concentration groups were evaluated. A detailed qualitative examination of the testes was conducted, taking into account the tubular stages of the spermatogenic cycle. A gross necropsy was conducted on the rats that were sacrificed due to moribund condition, and protocol-specified tissues were retained for histological examination.

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a. Detailed descriptions of all procedures used in the conduct of this study are provided in the appropriate sections of this report and in APPENDIX C (PROTOCOL).

1.2. Results

The test diets used for exposure were within ±15% of theoretical concentrations, homogeneous (RSD of ±5%) and stability was established for up to 8 weeks.

Consumed dosages (mg/kg/day)

Exposure Group (ppm)	100	1000	10000
DS 1 to 57			
Mean	10.9	109.3	1087.6
Range (DS)	8.0 (50-57) to 17.70 (8-15)	81.8 (50-57) to 180.7 (8-15)	807.2 (50-57) to 1784.6 (8-15)

DS - day of study

No mortality related to butylparaben occurred. Two rats, one rat in the 0 ppm exposure group and one rat in the 100 ppm exposure group, were sacrificed on DSs 32 and 44, respectively. These rats were sacrificed due to lesions of the eye resulting from retro-orbital bleeding.

No clinical and no necropsy observations or changes in body weight, body weight gain, feed consumption, organ weights, daily sperm production related to butylparaben occurred at exposure levels as high as 10000 ppm. Histological evaluation of reproductive organs, liver, adrenal glands, thyroid and pituitary gland also revealed no adverse findings. No differences considered related to the test substance occurred in testosterone, lutenizing hormone or follicle stimulating hormone levels.

1.3. Conclusion

On the basis of these data, the no-observable-effect-level (NOEL) for general toxicity including hormone levels for testosterone, LH and FSH, histopathology of reproductive organs, liver, adrenal glands, thyroid and pituitary and sperm analysis is 10000 ppm.

for 17 Mar 05
Raymond G. York, Ph.D., DABT Date
Associate Director of Research

17 MAR 05
Alan M. Hoberman, Ph.D., DABT Date
Director of Research and Study Director

2. DESCRIPTION OF TEST PROCEDURES

2.1. Conduct of Study

2.1.1. Sponsor

The Cosmetic, Toiletry and Fragrance Association (CTFA), 1101 17th Street NW Suite 300, Washington, D.C. 20036

2.1.2. Testing Facility

Charles River Discovery and Development Services (CR-DDS), Argus Division, 905 Sheehy Drive, Building A, Horsham, Pennsylvania 19044-1241

2.1.3. Study Number

1203-006

2.1.4. Purpose of the Study

The purpose of this study was to test for toxic effects/disturbances resulting from oral (diet) exposure to butylparaben of Crl:(WI) BR male rats on spermatogenesis.

2.1.5. Regulatory Compliance

The study was conducted in compliance with Good Laboratory Practice (GLP) regulations of the FDA[(1)]. Quality Assurance Unit findings derived from the inspections during the conduct of this study are documented and have been provided to the Study Director and the Testing Facility Management.

2.1.6. Ownership of the Study

The Sponsor owns the study. All raw data, analyses, reports and preserved tissues are the property of the Sponsor.

2.1.7. Study Monitor

Linda Loretz, Ph.D., DABT

2.1.8. Study Director

Alan M. Hoberman, Ph.D., DABT (Director of Research)

2.1.9. Technical Performance

2.1.9.1. CR-DDS

John F. Barnett, B.S. (Director of Operations, Argus Division)
Daniel A. Nolan, B.S., LATG (Manager Technical Training, Argus Division)
Rachel J. Koneski, B.S., ALAT (Team Leader, Argus Division)
Alethia Detweiler, B.S. (Necropsy Laboratory Technician, Argus Division)
Thomas E. McGoldrick, B.A. (Necropsy Team Leader, Argus Division)
Timothy M. Zeigenfuss, B.S. (Formulation Laboratory Technician, Argus Division)
Richard Norlin, M.S. (Principal Investigator, Worcester Division, Worcester, Massachusetts) - Concentration, Homogeneity and Stability Analyses of Butylparaben in the Test Diets
Julian Gulbinski, III, B.S., M.B.A. (Scientist and Principal Investigator, Argus Division) - Hormone Assays

2.1.9.2. Subcontractor Facilities

John J. Brodzinsky, Protameen Chemicals, Inc., Totowa, New Jersey) - Bulk Test Article Sampling
Walter Hogg (Southern Testing and Research Labs, a division of Microbac Laboratories, Inc., Wilson, North Carolina) - Diet Analysis for Phytoestrogens and total Parabens in the Diets prior to adding of Butylparaben
Peter Mann, D.V.M., Diplomate, ACVP (Principal Investigator, EPL, Inc., Sterling, Virginia) - Histological Evaluation

2.1.10. Report Preparation

Alan M. Hoberman, Ph.D., DABT
Larisa J. Wagner, B.S. (Study Coordinator)
Janessa L. Snodgrass, B.S. (Data Management Specialist)
Jo Anne M. Vico, B.S. (Supervisor of Data Management)
Rachel G. Statuti (Report Administrator)

2.1.11. Report Review

Valerie A. Sharper, M.S. (Senior Scientist)

2.1.12. Date Protocol Signed

10 June 2004

2.1.13. Dates of Technical Performance

Rat Arrival (Fo Generation Dams and Litters)	15 JUN 04
Acclimation Period	15 JUN 04 - 20 JUN 04
Exposure Period [Day 21 postpartum through a minimum 56-day exposure period, all exposures continued until sacrifice)	21 JUN 04 - 20 AUG 04
Scheduled Sacrifice - Fo Generation Female Rats	21 JUN 04 - 22 JUN 04
Scheduled Sacrifice - F1 Generation Pups	18 AUG 04 - 20 AUG 04

2.1.14. Records Maintained

The original report, raw data and reserve samples of the bulk test article and each lot of the carrier are retained in the archives of the Testing Facility. Any preserved tissues are retained in the archives of the Testing Facility for one year after the mailing of the draft final report, after which time the Sponsor will decide their final disposition. All unused prepared diets were discarded at the Testing Facility. Backup samples will be discarded at the Testing Facility following issue of the final report or at CR-DDS Worcester Division, Worcester, Massachusetts, upon acceptance of the analytical results. Disposition of the remaining bulk test article will be documented in the raw data. Tissues, blocks and slides will be archived at the Testing Facility.

2.2. Test Article Information

2.2.1. Description

Butylparaben - a white powder

2.2.2. Lot Number

B3140

2.2.3. Date Received and Storage Conditions

The test article was received on 1 April 2004 and was stored at room temperature.

2.2.4. Special Handling Instructions

Standard safety precautions (use of protective clothing, gloves, dust-mist/HEPA-filtered mask, safety goggles or safety glasses with side shields) were taken during diet preparation and exposure.

2.2.5. Analysis of Activity

Information to document or certify the identity, composition, strength and activity/purity of the test article was provided to the Testing Facility. Documentation of method of synthesis will not be provided as the test article was purchased. This deviation from the protocol did not affect the outcome of the study. A Certificate of Analysis is available in APPENDIX D. The expiration date is 10 February 2006.

2.3. Carrier Information

2.3.1. Descriptions, Lot Numbers, Dates Received, Storage Conditions, Supplier and Expiration Dates

Carrier	Description	Lot Number	Date Received	Storage Conditions	Supplier	Expiration Date
The meal form of CE-2 diet (CLEA Japan, Inc.)	Brown powder	E-2044AQYA	21 JUN 04	Room temperature	CLEA Japan, Inc.[a]	14 OCT 04
		E-2034-YA	28 MAY 04			03 NOV 04

a. CLEA Japan, Inc., Tokyo, Japan.

2.3.2. Special Handling Instructions

Standard safety precautions (use of protective clothing, gloves, dust-mist/HEPA-filtered mask, safety goggles or safety glasses with side shields) were taken during diet preparation and exposure.

2.3.3. Analysis of Purity

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to have been present in the carrier that would have interfered with the results of this study.

2.4. Reagent Information

2.4.1. Descriptions, Lot Numbers, Dates Received, Storage Conditions, Supplier and Expiration Dates

Reagent	Description	Lot Number	Date Received	Storage Conditions	Supplier	Expiration Date
Acetone	Clear, colorless liquid	H451 A10477	14 JUN 04	Room temperature, in a fireproof cabinet	Mallinckrodt Baker, Inc.[a]	JUN 08
Acetone (99.5%, A.C.S. Spectrophotometric Grade)	Clear liquid	00652AC	26 FEB 04	Room temperature	Sigma-Aldrich, Inc.[b]	JAN 08

a. Mallinckrodt Baker, Inc., Paris, Kentucky
b. Sigma-Aldrich, Inc., St. Louis, Missouri

2.4.2. Special Handling Instructions

Standard safety precautions (use of protective clothing, gloves, dust-mist/HEPA-filtered mask, safety goggles or safety glasses with side shields) were taken during diet preparation.

2.4.3. Analysis of Purity

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to have been present in the reagent that would have interfered with the results of this study.

2.5. Test Article Preparation and Storage Conditions

Formulations (diets) containing the test article were prepared at least once every two weeks at the Testing Facility, including once before initiation of the exposure period for a pre-study analysis. Prepared diets were stored at room temperature. Acetone was used only for the purpose of transferring the test article to the carrier; the acetone dissipated and was not a permanent component of the diet mixture.

2.5.1. Sample Information

Sample Type	Size	Dates Retained	Storage Conditions	Shipped To/Shipping Conditions	Date Shipped
Bulk Test Article[a]	2 g	01 APR 01 20 AUG 04	Room temperature	CR-DDS Worcester Division[b]/Ambient conditions	01 APR 04 24 AUG 04
Diet[c] E2034-YA E-2044AQYA E-2034-YR[d]	 500 g 125 g 1000 g	 01 JUN 04 28 JUN 04 27 APR 04	Room temperature	Southern Testing and Research Labs[e]/Ambient conditions	 01 JUN 04 28 JUN 04 27 APR 04
Homegeneity[f] (all levels)	25 g	15 JUN 04	Room temperature	CR-DDS Worcester Division[b]/Ambient conditions	15 JUN 04
Concentration[g] (all levels)	25 g	28 JUN 04 05 JUL 04	Room temperature	CR-DDS Worcester Division[b]/Ambient conditions	28 JUN 04 06 JUL 04
Stability[h]	25 g	15 JUN 04	Room temperature	CR-DDS Worcester Division[b]/Ambient conditions	15 JUN 04
Bulk Test Article Reserve	1 g	15 JUN 04	Room temperature	Testing Facility Archives	28 JUN 04
Carrier Reserve E2034-YA E-2044AQYA	125 g	 15 JUN 04 28 JUN 04	Room temperature	Testing Facility Archives	 28 JUN 04 26 AUG 04
Acetone Reserve 00652AC H451 A10477	5 mL	 15 JUN 04 28 JUN 04	Room temperature	Testing Facility Archives	 28 JUN 04 26 AUG 04

a. A sample of the bulk test article was retained on the last day of exposure. The sample was sent but was returned unanalyzed after completion of the study as noted in the protocol. This deviation did not affect the outcome of the study as the bulk test substance is known to be stable until Febuary 2006.
b. CR-DDS Worcester Division, Worcester, Massachusetts
c. A sample of each lot of carrier used on study was collected and shipped for analysis for phytoestrogen and paraben levels.
d. Lot E2034-YR of the diet was shipped to CR-DDS Worcester Division, Worcester, Massachusetts for analytical purposes but was not used for any diet formulations on the study because stability data of the butylparaben in the diet was established for 8 weeks, so no additional preparations were needed.
e. Southern Testing and Research Labs, Wilson, North Carolina
f. Homogeneity of the prepared diets was verified prior to the initiation of exposure. Duplicate samples were taken from the top, middle and bottom of each concentration to be used on study on the day of the prestudy preparation. One sample of each duplicate set was shipped for analysis; the remaining samples were retained at the Testing Facility as backup samples.
g. Concentration of the prepared diets was verified on the day of prestudy preparation. Duplicate samples were taken from the top, middle and bottom of each concentration to be used on study. Concentration of the prepared diets was also verified for each preparation after the prestudy preparation. Duplicate samples were taken from the middle of the preparations for all concentrations on each day of preparation during the study. For any diet analysis, one sample of each duplicate set was shipped for analysis; the remaining samples were retained at the Testing Facility as backup samples.

h. Stability of the prepared diets was verified during this study in conjunction with a prestudy preparation by analysis of samples collected from the lowest and highest concentrations used on this study after storage at room temperature for at least two, four and eight weeks. Triplicate samples were taken from the lowest and highest concentrations on the day of the prestudy preparation. All stability samples were shipped for analysis and stored at room temperature upon receipt. At the scheduled timepoints after the initial analysis (homogeneity results obtained during the prestudy analyses served as time zero), duplicate samples were analyzed. The remaining stability samples served as backup samples to be analyzed if the analytical results were not accepted from one of the stability analyses. The backup samples were discarded once the stability results were accepted.

2.5.2. Analytical Results

Acceptance criteria for analytical results for each group were defined as follows: 1) concentration results were considered acceptable if the difference between the mean value found and the targeted concentration was ≤15%; 2) homogeneity results were considered acceptable if the relative standard deviation (RSD) of the mean value at each sampling location was ≤5%; and 3) results of stability analysis were within ±10% of the concentration found during the corresponding initial concentration analysis.

2.6. Test System

2.6.1. Species

Rat

2.6.2. Strain

Crl:(WI) BR

2.6.3. Supplier (Source)

Charles River Laboratories, Inc., Raleigh, North Carolina

2.6.4. Sex

Weaned (starting at 22 days of age) male rats were exposed to the test article and/or the carrier.

Fo generation female rats were used only as breeders to produce the F1 generation and were not considered part of the Test System. The weight range for the Fo generation dams was 204 g to 275 g on the day after arrival at the Testing Facility.

2.6.5. Rationale for Test System

The Crl:(WI) BR rat was selected as the Test System because this strain of rat has been demonstrated to be sensitive to reproductive toxins and has been widely used throughout industry for reproductive toxicity evaluations.

2.6.6. Test System Data

Number of Male Rats Assigned	64
Date of Birth	01 JUN 04 - 03 JUN 04
Age at Arrival	13, 14, or 15 days postpartum
Weight (g) on First Day of Exposure	28.3 - 48.7

2.6.7. Method of Randomization

2.6.7.1. Fo Generation Female Rats/F1 Generation Litters

Female rats were naturally bred at the Supplier's facility by breeder male rats of the same source and strain. The day of delivery was designated day 1 of lactation (postpartum). The female rats were allowed to deliver their litters at the Supplier and shipped to arrive at the Testing Facility on days 13, 14 or 15 postpartum. Day 1 was the day of birth. Upon arrival, dams were placed into nesting boxes in consecutive order, by day postpartum.

2.6.7.2. F1 Generation Male Rats

Day 1 of lactation (postpartum) is defined as the day of birth.

On the day of randomization, a table of random units was used to select eight litters from nine possible litters. One litter was withheld from selection for possible future use on study. From the eight litters selected, all male rats were assigned to dosage groups as follows: the first and fifth male rats were assigned to Group I, the second and sixth male rats were assigned to Group II, the third and seventh male rats were assigned to Group III and the fourth and eighth male rats were assigned to Group IV.

A table of random units was used to select six male rats per group for histopathological evaluation of the liver, adrenal, thyroid and pituitary glands. These tissues were collected from the remaining rats in each group and frozen for possible hormone analysis.

2.6.8. System of Identification

2.6.8.1. Fo Generation Female Rats

Female rats were assigned temporary animal numbers on the day after receipt and identified with a permanent marker.

2.6.8.2. F1 Generation Male Rats

On the day of study assignment, pups were given unique permanent identification numbers using a tattoo (AIMS Animal Identification and Marking System, AIMS, Inc., Piscataway, New Jersey with AIMS Black Pigment).

Cage tags were marked with the study number, permanent rat number, sex, generation test article identification, group number and dosage level.

2.7. Husbandry

2.7.1. Research Facility Registration

USDA Registration No. 14-R-0144 under the Animal Welfare Act, 7 U.S.C. 2131 *et seq*.

2.7.2. Study Room

The study room was maintained under conditions of positive airflow relative to a hallway and independently supplied with a minimum of ten changes per hour of 100% fresh air that had been passed through 99.97% HEPA filters. Room temperature and humidity were monitored constantly throughout the study. Room temperature was targeted at 64°F to 79°F (18°C to 26°C); relative humidity was targeted at 30% to 70%[a].

2.7.3. Housing

All cage sizes and housing conditions were in compliance with the *Guide for the Care and Use of Laboratory Animals*[(2)].

2.7.3.1. Fo Generation Female Rats/F1 Generation Litters

Each dam and delivered litter was housed in a common nesting box during the postpartum period.

2.7.3.2. F1 Generation Male Rats

After weaning, F1 generation male rats were individually housed in stainless steel, wire-bottomed cages.

2.7.4. Light

An automatically controlled 12-hours light:12-hours dark fluorescent light cycle was maintained. Each dark period began at 1900 hours.

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a. See APPENDIX F (TEMPERATURE AND RELATIVE HUMIDITY REPORTS).

2.7.5. Sanitization

2.7.5.1. Fo Generation Female Rats/F1 Generation Litters

During the postpartum period, bedding was changed as often as necessary to keep the rats dry and clean.

2.7.5.2. F1 Generation Male Rats

Cage pan liners were changed at least three times weekly. Cages were changed approximately every other week.

2.7.6. Diet

Rats were given either CE-2 diet (CLEA Japan, Inc., Tokyo, JAPAN) only (carrier control group) or test diets prepared using CE-2 diet and the test article. These diets were available *ad libitum* from individual feeders

2.7.7. Diet Analysis

A sample (125g for Lot E-2044AQYA and 500g for Lot E-2044-YA) of each lot of carrier (without test substance, butylparaben) used on study was collected and shipped for analysis for background phytoestrogen (total isoflavones, including Diadzin, Glycitin, Genistin, Daidzein, Glycitein, Genistein) and total paraben levels to an independent subcontractor facility (Southern Testing and Research Labs, Wilson, North Carolina). Copies of the results of the diet analyses are available in the raw data and in APPENDIX G.

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to have been present in the certified diet that would have interfered with the results of this study.

2.7.8. Water

Local water that had been processed by passage through a reverse osmosis membrane (R.O. water) was available to the rats *ad libitum* from an automatic watering access system and/or individual water bottles attached to the cages. Chlorine was added to the processed water as a bacteriostat.

2.7.9. Water Analysis

The processed water is analyzed twice annually for possible chemical contamination (Lancaster Laboratories, Lancaster, Pennsylvania) and monthly for possible bacterial contamination (QC Laboratories, Southampton, Pennsylvania). Copies of the results of the water are available in the raw data.

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to have been present in the drinking water that would have interfered with the results of this study.

2.7.10. Bedding Material

Bed-o'cobs® bedding (The Andersons Industrial Products Group, Maumee, Ohio) was used as the nesting material.

2.7.11. Bedding Analysis

Each lot of bedding is analyzed for possible contamination (Lancaster Laboratories, Lancaster, Pennsylvania). Copies of the results of the bedding analyses are available in the raw data.

Neither the Sponsor nor the Study Director was aware of any potential contaminants likely to have been present in the bedding that would have interfered with the results of this study.

2.8. Methods

2.8.1. Dosage Administration

Group	Concentration (ppm)	Number of Male Rats	Assigned Rat Numbers
I	0	16	12737 - 12752
II	100	16	12753 - 12768
III	1000	16	12769 - 12784
IV	10000	16	12785 - 12800

The test substance was considered 100% pure for the purpose of dosage calculations.

2.8.2. Rationale for Dosage Selection

Dosages were selected by the Sponsor on the basis of previous studies conducted with the test article.

2.8.3. Route and Rationale for Route of Administration

The oral route via the diet was selected for use because it is one possible route of human exposure.

2.8.4. Method and Frequency of Administration

A constant concentration of the test article in the diet was offered to the male rats in each group, and the mg/kg/day dosages consumed were calculated and presented for periods corresponding to body weight and feed consumption observations.

A carrier control and three test diet concentrations were given to the rats. Rats (in Groups II through IV) were given continual access to the test article in the diet for at least 56 days beginning on day 21 postpartum. All exposures were continued to the day of sacrifice.

2.8.5. Method of Study Performance

2.8.5.1. Fo Generation Female Rats

Rats were observed for viability at least twice each day of the study. These rats were also examined for clinical observations and general appearance at least once. Maternal behavior was recorded daily beginning the day after arrival at the Testing Facility. Body weights were recorded on the day after arrival and before sacrifice (terminal weight). Feed consumption was monitored as feed was replenished on an as-needed basis.

2.8.5.2. F1 Generation Male Rats

Rats were observed for viability at least twice each day of the study. These rats were also examined for clinical observations and general appearance daily (recorded by litter) during the acclimation period and daily during the exposure period. Body weights were recorded once during the acclimation period, daily during the exposure period and at sacrifice (terminal weight). Feed consumption values were recorded twice weekly during the exposure period.

Beginning at the start of week 3 of the exposure period, blood samples (at least 1.6 mL each) were collected bi-weekly (every other week) from each male rat assigned to study. The time of each blood collection was recorded in the raw data. Blood samples were collected at approximately the same time each week of collection (standardized for time of day, between 8:30 am and 11:00 am EDT) to address the circadian, pulsatile release of male hormones. Blood was collected from the orbital sinus. The rats were anesthetized using isoflurane/oxygen before sample collection. The samples were transferred into serum separator tubes and spun in a centrifuge. The resulting serum (0.8 mL) was transferred into polypropylene tubes labeled with the protocol number, rat number, group number, dosage level, day of study, collection interval, date of collection, species, generation and storage conditions. All samples were immediately frozen on dry ice and maintained frozen (approximately -80°C) until analysis at the Testing Facility. Samples were analyzed at the Testing Facility for LH (luteinizing hormone), FSH (follicle-stimulating hormone) and testosterone. All hormones were analyzed using Enzyme Linked Immunosorbent Assay (ELISA) methodology (Amersham Pharmacia Biotech Ltd. - Rat Lutenizing Hormone - Catalog number RPN 2562; Amersham Pharmacia Biotech Ltd. - Rat Follicle Stimulating Hormone - Catalog number RPN 2560 and Biomeda - Testosterone - Catalog number EU 1048).

2.8.6. Gross Necropsy

2.8.6.1. Necropsy - Fo Generation Female Rats

On day 21 postpartum, female rats with litters assigned to study were sacrificed by carbon dioxide asphyxiation and discarded without further evaluation.

Dams and litters not assigned to the study were sacrificed after pups were selected for study assignment and it was determined that no additional pups were needed. Carcasses were discarded without further evaluation.

2.8.6.2. Necropsy - F1 Generation Male Rats

On the day of sacrifice, all surviving F1 generation male rats were sacrificed by carbon dioxide asphyxiation, and blood samples (at least 2 mL each) for possible future analysis for butylparaben and para hydroxy benzoic acid levels were collected from the vena cava of each male rat assigned to study. The whole blood samples were transferred into EDTA-coated (purple-top) tubes and spun in a centrifuge. The resulting plasma was transferred into polypropylene tubes labeled with the protocol number, rat number, group number, dosage level, day of study, collection interval, date of collection, species, generation and storage conditions. All samples were immediately frozen on dry ice and maintained frozen (approximately -80°C) for analysis.

A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Gross lesions were retained in neutral buffered 10% formalin for possible future evaluation. A table of random units was used to select one male control group rat from which all tissues examined at necropsy were retained, in order to provide control tissues for potential comparative histopathological evaluations. To assess the potential toxicity of the test article on the male reproductive system, reproductive organs were weighed and retained for possible histological evaluation and sperm evaluations were conducted.

The following organs were individually weighed: liver, adrenal glands (paired), thyroid, pituitary, right testis, left testis, left epididymis (whole and cauda), right epididymis, seminal vesicles (with and without fluid) and prostate (ventral and dorsal). The left testis from each rat was collected for evaluation of Daily Sperm Production (DSP) determinations (i.e. testicular spermatid concentration).

The liver, adrenal, thyroid and pituitary glands from ten male rats per exposure group were quick-frozen in liquid nitrogen and stored at approximately -80°C for possible hormone measurements.

Sperm concentration and motility were evaluated using computer-assisted sperm analysis (CASA). Motility was evaluated by the Hamilton Thorne Integrated Visual Optical System (IVOS), using a sample collected from the left vas deferens. A homogenate was prepared from the left cauda epididymis for evaluation by the Hamilton Thorne IVOS to determine sperm concentration (sperm per gram of tissue weight). The

remaining portion of the left cauda epididymis was used to manually evaluate sperm morphology for determination of the percentage of normal sperm in a sample of at least 200 and for qualitative evaluation of abnormal sperm, including such categories as abnormal head, abnormal tail, and abnormal head and tail.

The left testis was used to evaluate testicular spermatid concentration via CASA. The left testis was weighed both before and after removal of the tunica albuginea and then homogenized. A sample of the resulting homogenate was stained with an IDENT Stain Kit from Hamilton Thorne Research, and a slide was prepared for analysis by the Hamilton Thorne IVOS. Ten fields were analyzed to determine testicular spermatid concentration (spermatids per gram of tissue weight). All images produced during analysis were retained as raw data.

The remaining portion of the left epididymis (corpus and caput), the right epididymis, prostate and seminal vesicles were retained in neutral buffered 10% formalin for possible histopathological evaluation. The right testis was fixed in modified Davidson's solution for 24 to 48 hours and then retained in neutral buffered 10% formalin for histopathological evaluation[3]. The liver, adrenal, thyroid and pituitary glands from six male rats per exposure group were fixed in neutral buffered 10% formalin for possible histopathological evaluation.

Tissues to be examined histologically were shipped to EPL, Inc., Sterling, Virginia, USA, routinely processed, embedded in paraffin, sectioned at 5 microns and stained with hematoxylin and eosin. Histological examination was performed on the reproductive organs (remaining portions of the left epididymis, right epididymis, right testis, prostate and seminal vesicles) from all control and high test article concentration group rats. Additionally, the liver, adrenals, thyroid and pituitary glands from six rats in the control and high test article concentration groups were evaluated. A detailed qualitative examination of the testes was conducted, taking into account the tubular stages of the spermatogenic cycle. The examination was conducted in order to identify treatment-related effects such as missing germ cell layers or types, retained spermatids, multinucleate or apoptotic germ cells and sloughing of spermatogenic cells into the lumen. Any cell- or stage-specificity of testicular findings were noted[4]. Histopathological evaluations were conducted by a Board Certified Veterinary Pathologist with expertise in reproductive pathology. Results of the histopathological evaluation are available in APPENDIX H.

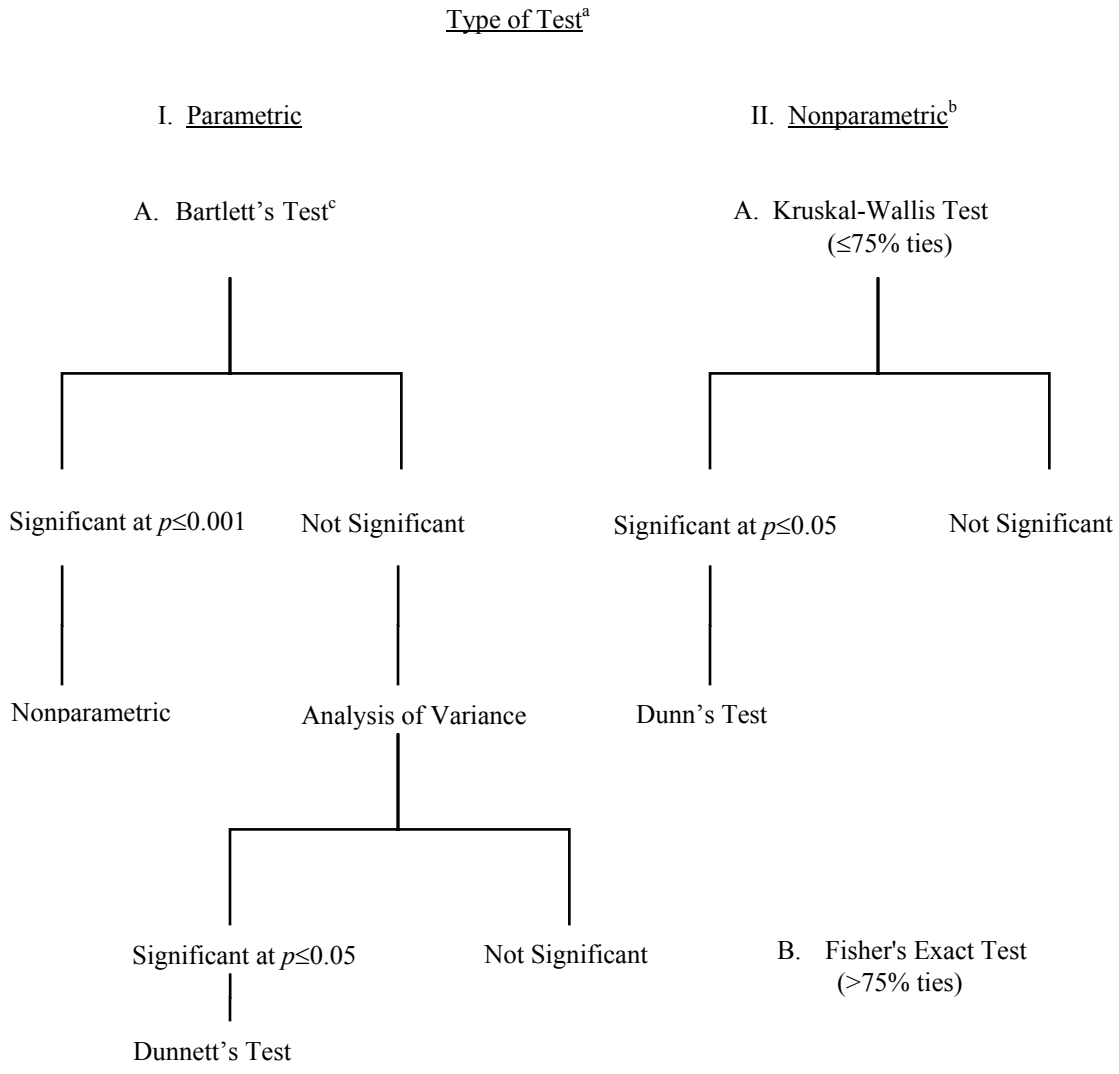
Rats that were sacrificed due to moribund condition were examined for the cause of moribund condition on the day the observation was made. Gross necropsy included an initial physical examination of the external surfaces and all orifices, as well as internal examination of tissues and organs *in situ*. In addition, an examination of the cranial, thoracic and abdominal cavities was conducted. The testes and epididymides were excised and paired organ weights were recorded. The following tissues or representative samples were retained in neutral buffered 10% formalin for possible future histological examination: adrenal glands, aorta, bone marrow (sternum), brain (cerebrum, cerebellum, medulla/pons), epididymides, esophagus, eyes (with optic nerve), femur,

heart, large intestine (colon, cecum, rectum), small intestines (duodenum, jejunum, ileum), kidneys, liver, lungs, lymph nodes (mandibular, mesenteric), pancreas, Peyer's Patches, pituitary, prostate, salivary gland (mandibular), sciatic nerve, seminal vesicles, skeletal muscle, skin, spinal cord (cervical, mid-thoracic, lumbar), spleen, stomach, testes, thymus, thyroid/parathyroid, trachea and urinary bladder. The testes were fixed in modified Davidson's solution for 24 to 48 hours and then retained in neutral buffered 10% formalin. The lungs were perfused with neutral buffered 10% formalin. Gross lesions were retained in neutral buffered 10% formalin and examined histologically.

2.8.7. Data Collection and Statistical Analyses

Data generated during the course of this study were recorded either by hand or using the *Argus Automated Data Collection and Management System*, the *Vivarium Temperature and Relative Humidity Monitoring System* and the *Hamilton Thorne IVOS*. All data were tabulated, summarized and/or statistically analyzed using the *Argus Automated Data Collection and Management System*, the *Vivarium Temperature and Relative Humidity Monitoring System, Microsoft® Excel* (part of Microsoft® Office 97/2000/XP), Quattro Pro 8 and/or *The SAS System* (version 6.12).

Averages and percentages were calculated. The following schematic represents the statistical analyses of the data:



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a. Statistically significant probabilities are reported as either $p \leq 0.05$ or $p \leq 0.01$.
b. Proportion data are not included in this category.
c. Test for homogeneity of variance.

Clinical observations and other proportional data were analyzed using the Variance Test for Homogeneity of the Binomial Distribution[5].

Continuous data (e.g., body weights, body weight changes, organ weights and feed consumption values) were analyzed using Bartlett's Test of Homogeneity of Variances[6] and the Analysis of Variance[7], when appropriate [i.e., Bartlett's Test was not significant ($p>0.001$)]. If the Analysis of Variance was significant ($p\leq0.05$), Dunnett's Test[8] was used to identify the statistical significance of the individual groups. If the Analysis of Variance was not appropriate [i.e., Bartlett's Test was significant ($p\leq0.001$)], the Kruskal-Wallis Test[9] was used (≤75% ties). In cases where the Kruskal-Wallis Test was statistically significant ($p\leq0.05$), Dunn's Method of Multiple Comparisons[10] was used to identify the statistical significance of the individual groups. If there were greater than 75% ties, Fisher's Exact Test[11] was used to analyze the data.

Count data were evaluated using the procedures described above for the Kruskal-Wallis Test.

Sperm motility data were expressed as percentages and analyzed, as indicated above, by parametric and nonparametric methods.

3. RESULTS

3.1. Analytical Results (APPENDIX E and G)

3.1.1. Test Substance

The test diets used for exposure were within ±15% of theoretical concentrations. Diets were homogeneous with an RSD of ±5%, and stability was established for up to 8 weeks.

3.1.2. Total Parabens and Phytoestrogens

Portions of the test diets without any added test substance were analyzed for total parabens and phytoestrogens. The level of para-aminobenzoic acid (PABA) which is a measure of the total parabens in the diet was 6.92 and 4.37 ppm for the two lots of diets used on the study. The level of phytoestrogens (total isoflavones, including Diadzin, Glycitin, Genistin, Daidzein, Glycitein, Genistein) in the diets was 912 and 924 ppm for the same respective lots.

3.2. Consumed Dosages (Summary - Table 1)

Consumed dosages (mg/kg/day)

Exposure Group (ppm)	100	1000	10000
DS 1 to 57			
Mean	10.9	109.3	1087.6
Range (DS)	8.0 (50-57) to 17.70 (8-15)	81.8 (50-57) to 180.7 (8-15)	807.2 (50-57) to 1784.6 (8-15)

DS - day of study

Exposures for the rats gradually decreased on a mg/kg/day basis over the course of the study from day 8 of the study (DS 8), with lower levels of exposure occurring later in the exposure period. These differences in dosages over the course of the study are the result of feeding a constant ppm of test article in the diet to rats at different ages (i.e., weights). The younger, and therefore lighter, rats are exposed to a higher level of test substance on a mg/kg/day basis than older, and therefore heavier, rats.

3.3. Mortality and Clinical Observations (Summary - Table 2; Individual Data - Table 15)

3.3.1. Mortality

No mortality related to butylparaben occurred. Two rats, one rat in the 0 ppm exposure group and one rat in the 100 ppm exposure group, were sacrificed on DSs 32 and 44, respectively. These rats were sacrificed due to lesions of the eye resulting from retro-

orbital bleeding. Each rat is described below. All other rats survived to scheduled sacrifice.

Rat 12741 in the 0 ppm exposure group was sacrificed due to its moribund condition on DS 32. This rat had a traumatized cornea, exophthalmos, dried cornea and traumatized conjunctiva on DS 30 to 32, corneal ulceration and desiccation and a swollen head on DS 31 to 32 and a collapsed anterior chamber of the right eye on DS 32. These observations were all secondary to retro-orbital bleeding. The rat generally gained weight until sacrifice and feed consumption values were comparable to the other rats in the exposure group. With the exception of the lesions in the eye, all other tissues appeared normal at necropsy.

Rat 12753 in the 100 ppm exposure group was sacrificed due to its moribund condition on DS 44. This rat had a scab in the left axilla on DS 31 to 41, vocalization, exophthalmos, red periorbital substance, unreactive pupil of the right eye, periorbital discoloration, head tilt and a swollen head on DS 44. The observations on DS 44 were all secondary to retro-orbital bleeding. The rat generally gained weight until sacrifice and feed consumption values were comparable to the other rats in the exposure group. All tissues appeared normal.

3.3.2. Clinical Observations

Urine-stained abdominal fur occurred in three rats and sparse coat occurred in two rats of the 16 rats in the 10000 ppm exposure group. These clinical observations were not considered related to the test substance even though they occurred in the highest exposure group because urine-stained abdominal fur also occurred in one rat in the control group, sparse hair coat also occurred in one rat in the 100 ppm exposure group, the observations did not persist and these observations occur sporadically in rats at this Testing Facility.

No other clinical observations related to butylparaben occurred at exposure levels as high as 10000 ppm. The clinical observations that did occur were not considered related to the test substance because: 1) the incidence was not dosage dependent; 2) the observation occurred in a rat that was sacrificed due to problem with retro-orbital bleeding; and/or 3) the observation was secondary to retro-orbital bleeding. These observations included chromorhinorrhea; chromodacryorrhea; a scab or abrasion on the head, neck, mouth, nose and/or left axilla; localized alopecia; dental problems; lacrimation; excess salivation; enlarged eye; corneal opacity; ulceration and bent tail.

3.4. Body Weights and Body Weight Changes (Figure 1; Summaries - Tables 3 and 4; Individual Data - Table 16)

Body weights and body weight gains were not affected by exposure to butylparaben as high as 10000 ppm. No statistically significant differences occurred among the groups.

Body weights as a percent of the control value

Exposure Group (ppm)	100	1000	10000
DS 59 %	97.4	103.9	100.0

DS- day of study

3.5. Absolute (g/day) and Relative (g/kg/day) Feed Consumption Values (Summaries - Tables 5 and 6; Individual Data - Table 17)

Absolute and relative feed consumption values were not affected by exposure to butylparaben as high as 10000 ppm.

Absolute feed consumption values were significantly reduced ($p \leq 0.05$ or $p \leq 0.01$) on DS 1 to 8 in the 100 and 10000 ppm exposure groups. These reductions were not considered related to the test substance because the reductions were not dose dependent, none persisted and no effect on relative feed consumption occurred.

3.6. Necropsy Observations (Summary - Table 7; Individual Data - Table 18)

No necropsy observations related to butylparaben occurred.

3.7. Terminal Body Weights, Organ Weights and Ratios (%) of Organ Weight to Terminal Body Weights (Summaries - Tables 8 and 9; Individual Data - Table 19)

Terminal body weights, organ weights and the ratio of these organ weights to the terminal body weight were not affected by exposure to butylparaben as high as 10000 ppm. No statistically significant differences occurred among the groups.

3.8. Sperm Evaluation (Summaries - Tables 10 and 11; Individual Data - Tables 20 and 21)

Exposure to butylparaben at levels as high as 10000 ppm did not affect sperm motility, sperm count, morphology or daily sperm production.

3.9. Histopathology (Appendix H)

Exposure to butylparaben at levels as high as 10000 ppm did not produce any adverse findings in the reproductive organs or the liver, adrenal glands, thyroid, or pituitary.

3.10. Hormone Assays (Summaries - Tables 12, 13 and 14; Individual Tables 22, 23 and 24)

No differences in testosterone, lutenizing hormone (LH) or follicle stimulating hormone (FSH) levels were considered related to exposure to the test diets.

Statistically significant reductions ($p \leq 0.05$ to $p \leq 0.01$) in the levels of testosterone occurred in the 1000 and 10000 ppm exposure groups after three weeks of exposure to test diets. At the end of exposure significant increases ($p \leq 0.05$ to $p \leq 0.01$) occurred in the 10000 ppm exposure group for testosterone and FSH, relative to the control group value. These changes were not considered related to the test substance. The reductions that occurred after week 3 were the result of two high values in the control group male rats. The expected general increase in testosterone and FSH levels occurred in each group over the eight week study.

Significant reductions ($p \leq 0.01$) in lutenizing hormone levels that occurred after five weeks of exposure in the 100 and 10000 ppm exposure groups were not considered related to butylparaben, because the reductions occurred only at one time point and were not dosage-dependent.

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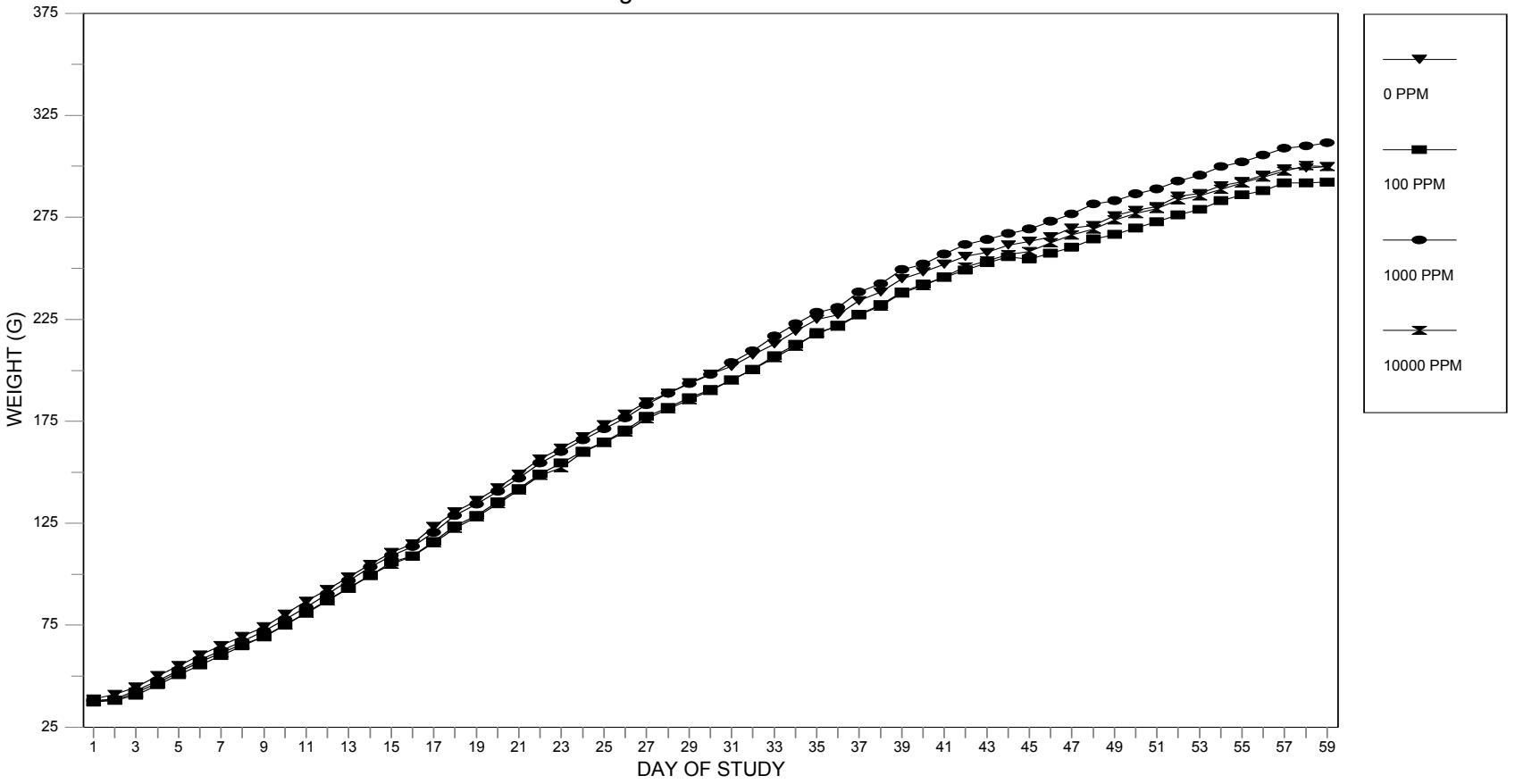
APPENDIX A

REPORT FIGURE

BODY WEIGHTS - MALE RATS

Figure 1



APPENDIX B

REPORT TABLES

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 1 (PAGE 1): CONSUMED DOSAGES (MG/KG) - SUMMARY - MALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (PPM)a		0	100	1000	10000
RATS TESTED		16	16	16	16
DIETARY DOSAGE (MG/KG)					
DAYS 1 - 8	MEAN±S.D.	0.0 ± 0.0	17.0 ± 1.2	177.5 ± 12.1	1705.5 ± 168.1
DAYS 8 - 15	MEAN±S.D.	0.0 ± 0.0	17.7 ± 1.1	180.7 ± 16.7	1784.6 ± 165.1
DAYS 15 - 22	MEAN±S.D.	0.0 ± 0.0	14.6 ± 0.7	145.8 ± 9.5	1446.7 ± 116.4
DAYS 22 - 29	MEAN±S.D.	0.0 ± 0.0	13.3 ± 0.7	131.8 ± 9.8	1341.4 ± 117.3
DAYS 29 - 36	MEAN±S.D.	0.0 ± 0.0 [14]b,c	10.8 ± 0.5	109.3 ± 8.7	1089.6 ± 84.3
DAYS 36 - 43	MEAN±S.D.	0.0 ± 0.0 [14]b,c	9.8 ± 0.6	97.7 ± 9.9	989.6 ± 85.7
DAYS 43 - 50	MEAN±S.D.	0.0 ± 0.0 [15]c	8.1 ± 0.6 [15]b	81.9 ± 5.8 [15]b	838.5 ± 61.7 [15]b
DAYS 50 - 57	MEAN±S.D.	0.0 ± 0.0 [15]c	8.0 ± 0.4 [14]b,d	81.8 ± 10.4	807.2 ± 51.9 [15]b
DAYS 1 - 57	MEAN±S.D.	0.0 ± 0.0 [15]c	10.9 ± 0.4 [14]b,d	109.3 ± 8.2	1087.6 ± 67.8 [15]b

DAYS = DAYS OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Access to test diet occurred from day 1 of study until sacrifice.
b. Excludes values that were associated with spillage.
c. Excludes rat 12741, which was moribund sacrificed on day 32 of study.
d. Excludes rat 12753, which was moribund sacrificed on day 44 of study.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 2 (PAGE 1): CLINICAL OBSERVATIONS - SUMMARY - MALE RATS

DOSAGE GROUP	I	II	III	IV
DOSAGE (PPM)a	0	100	1000	10000
MAXIMUM POSSIBLE INCIDENCE	928/ 16	939/ 16	955/ 16	957/ 16
MORIBUND SACRIFICED	1b	1c	0	0
CHROMORHINORRHEA	13/ 5	33/ 6	6/ 3	17/ 3
CHROMODACRYORRHEA	21/ 5	25/ 4	4/ 2	16/ 3
URINE-STAINED ABDOMINAL FUR	2/ 1	0/ 0	0/ 0	5/ 3
SPARSE COAT	0/ 0	1/ 1	0/ 0	7/ 2
BACK, HEAD, NECK, NOSE, MOUTH, AND/OR LEFT AXILLA: SCAB	37/ 3	36/ 4c	6/ 2	20/ 1
LOCALIZED ALOPECIA: TOTAL	0/ 0	18/ 2	4/ 1	18/ 1
NECK	0/ 0	0/ 0	4/ 1	18/ 1
LIMB(S)	0/ 0	14/ 1	0/ 0	17/ 1
HEAD	0/ 0	4/ 1	0/ 0	0/ 0
INCISOR(S): TOTAL	61/ 3	0/ 0	0/ 0	36/ 1
MISSING/BROKEN	7/ 2	0/ 0	0/ 0	25/ 1
MISALIGNED	54/ 1	0/ 0	0/ 0	36/ 1
LACRIMATION	0/ 0	0/ 0	0/ 0	1/ 1
EXCESS SALIVATION	0/ 0	0/ 0	0/ 0	1/ 1
BACK OR NECK: ABRASION	2/ 1	0/ 0	3/ 1	0/ 0
RIGHT EYE: TRAUMATIZED CORNEA	3/ 1b	0/ 0	2/ 1	0/ 0
HEAD TILT	0/ 0	28/ 2c	0/ 0	0/ 0
RIGHT OR LEFT EYE: ENLARGED	97/ 2	4/ 1	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Access to test diet occurred from day 1 of study until sacrifice.
b. Rat 12741 was moribund sacrificed on day 32 of study.
c. Rat 12753 was moribund sacrificed on day 44 of study.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 2 (PAGE 2): CLINICAL OBSERVATIONS - SUMMARY - MALE RATS

DOSAGE GROUP DOSAGE (PPM)a	I 0	II 100	III 1000	IV 10000
MAXIMUM POSSIBLE INCIDENCE	928/ 16	939/ 16	955/ 16	957/ 16
MORIBUND SACRIFICED	1b	1c	0	0
LEFT OR RIGHT EYE: CORNEAL OPACITY	17/ 1	2/ 1	0/ 0	0/ 0
BACK OR MOUTH: ULCERATION	1/ 1	2/ 1	0/ 0	0/ 0
RIGHT EYE: EXOPHTHALMOS	3/ 1b	1/ 1c	0/ 0	0/ 0
HEAD: SWOLLEN	2/ 1b	1/ 1c	0/ 0	0/ 0
TAIL BENT	0/ 0	28/ 1	0/ 0	0/ 0
VOCALIZATION	0/ 0	1/ 1c	0/ 0	0/ 0
RIGHT EYE: RED PERIORBITAL SUBSTANCE	0/ 0	1/ 1c	0/ 0	0/ 0
RIGHT EYE: UNREACTIVE PUPIL	0/ 0	1/ 1c	0/ 0	0/ 0
RIGHT EYE: PERIORBITAL DISCOLORATION	0/ 0	1/ 1c	0/ 0	0/ 0
RIGHT EYE: DRIED CORNEA	3/ 1b	0/ 0	0/ 0	0/ 0
RIGHT EYE: TRAUMATIZED CONJUCTIVA	3/ 1b	0/ 0	0/ 0	0/ 0
RIGHT EYE: CORNEAL DESSICATION	2/ 1b	0/ 0	0/ 0	0/ 0
RIGHT EYE: CORNEAL ULCERATION	2/ 1b	0/ 0	0/ 0	0/ 0

STATISTICAL ANALYSES OF CLINICAL OBSERVATION DATA WERE RESTRICTED TO THE NUMBER OF RATS WITH OBSERVATIONS.
MAXIMUM POSSIBLE INCIDENCE = (DAYS x RATS)/NUMBER OF RATS EXAMINED PER GROUP.
N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF RATS WITH OBSERVATION.
a. Access to test diet occurred from day 1 of study until sacrifice.
b. Rat 12741 was moribund sacrificed on day 32 of study.
c. Rat 12753 was moribund sacrificed on day 44 of study.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 3 (PAGE 1): BODY WEIGHTS - SUMMARY - MALE RATS

DOSAGE GROUP			I	II	III	IV
DOSAGE (PPM)a			0	100	1000	10000
RATS TESTED		N	16	16	16	16
BODY WEIGHT (G)						
DAY 1		MEAN±S.D.	38.9 ± 3.4	37.4 ± 4.2	38.0 ± 4.6	38.2 ± 5.2
DAY 2		MEAN±S.D.	41.0 ± 4.0	38.3 ± 5.3	38.8 ± 5.1	38.3 ± 5.5
DAY 3		MEAN±S.D.	44.7 ± 4.2	40.7 ± 6.4	42.8 ± 5.6	41.9 ± 5.9
DAY 4		MEAN±S.D.	50.2 ± 4.8	45.9 ± 6.5	47.8 ± 6.8	46.8 ± 6.9
DAY 5		MEAN±S.D.	55.3 ± 5.1	50.8 ± 6.6	53.0 ± 7.0	52.0 ± 7.9
DAY 6		MEAN±S.D.	60.4 ± 5.2	55.4 ± 7.0	58.2 ± 7.5	56.9 ± 8.5
DAY 7		MEAN±S.D.	65.0 ± 5.6	60.0 ± 7.5	62.5 ± 7.8	61.4 ± 9.1
DAY 8		MEAN±S.D.	69.5 ± 5.7	64.9 ± 7.6	67.0 ± 8.1	65.7 ± 9.4
DAY 9		MEAN±S.D.	74.1 ± 5.9	69.8 ± 8.0	72.1 ± 8.8	69.3 ± 10.1
DAY 10		MEAN±S.D.	80.4 ± 6.0	75.2 ± 8.5	77.7 ± 9.9	75.2 ± 10.9
DAY 11		MEAN±S.D.	86.8 ± 6.5	81.2 ± 9.7	83.9 ± 10.2	80.9 ± 11.1
DAY 12		MEAN±S.D.	92.6 ± 6.3	87.5 ± 10.6	90.4 ± 10.9	86.9 ± 11.8
DAY 13		MEAN±S.D.	98.6 ± 6.7	93.2 ± 11.0	96.7 ± 11.7	93.2 ± 12.3
DAY 14		MEAN±S.D.	104.8 ± 7.9	99.3 ± 11.8	103.5 ± 11.9	99.4 ± 12.7
DAY 15		MEAN±S.D.	110.7 ± 8.2	106.0 ± 12.9	109.2 ± 12.5	104.9 ± 13.7
DAY 16		MEAN±S.D.	114.8 ± 8.6	108.9 ± 12.9	113.6 ± 12.9	108.9 ± 13.8
DAY 17		MEAN±S.D.	123.4 ± 8.7	116.0 ± 13.1	120.5 ± 13.8	115.3 ± 14.2
DAY 18		MEAN±S.D.	130.5 ± 9.7	123.6 ± 13.8	128.7 ± 14.3	122.6 ± 15.1
DAY 19		MEAN±S.D.	136.1 ± 9.3	128.8 ± 13.9	134.4 ± 14.8	128.2 ± 15.2

DAY = DAY OF STUDY
a. Access to test diet occurred from day 1 of study until sacrifice.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 3 (PAGE 2): BODY WEIGHTS - SUMMARY - MALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (PPM)a		0	100	1000	10000
RATS TESTED	N	16	16	16	16
BODY WEIGHT (G)					
DAY 20	MEAN±S.D.	142.4 ± 11.2	135.5 ± 14.1	140.6 ± 15.0	134.6 ± 14.7
DAY 21	MEAN±S.D.	149.2 ± 11.3	142.0 ± 14.8	147.2 ± 14.3	141.2 ± 14.9
DAY 22	MEAN±S.D.	156.4 ± 11.6	149.2 ± 15.1	154.5 ± 15.0	148.4 ± 16.1
DAY 23	MEAN±S.D.	161.8 ± 11.3	154.4 ± 14.9	160.0 ± 15.8	152.3 ± 16.1
DAY 24	MEAN±S.D.	167.5 ± 12.3	160.3 ± 16.3	166.0 ± 15.7	159.8 ± 16.2
DAY 25	MEAN±S.D.	173.2 ± 13.2	164.7 ± 16.0	171.2 ± 15.1	164.6 ± 16.9
DAY 26	MEAN±S.D.	178.5 ± 13.3	170.6 ± 16.6	176.7 ± 16.3	169.8 ± 17.7
DAY 27	MEAN±S.D.	184.5 ± 14.0	177.2 ± 18.0	183.1 ± 16.5	176.2 ± 17.2
DAY 28	MEAN±S.D.	188.6 ± 14.6	181.7 ± 18.4	188.6 ± 16.7	181.0 ± 17.6
DAY 29	MEAN±S.D.	193.8 ± 14.5	186.4 ± 18.6	193.5 ± 17.4	185.6 ± 18.8
DAY 30	MEAN±S.D.	198.1 ± 15.0	190.5 ± 19.5	197.8 ± 17.1	190.0 ± 19.1
DAY 31	MEAN±S.D.	201.8 ± 15.7	195.1 ± 19.5	203.6 ± 18.1	195.2 ± 18.9
DAY 32	MEAN±S.D.	207.6 ± 15.6	200.4 ± 19.5	209.5 ± 18.9	200.4 ± 19.7
DAY 33	MEAN±S.D.	212.8 ± 16.7 [15]b	207.1 ± 20.3	216.6 ± 19.4	206.1 ± 19.7
DAY 34	MEAN±S.D.	219.1 ± 17.6 [15]b	212.6 ± 21.2	222.7 ± 19.8	211.7 ± 19.4
DAY 35	MEAN±S.D.	224.9 ± 16.9 [15]b	217.7 ± 21.2	228.4 ± 20.6	218.4 ± 21.5
DAY 36	MEAN±S.D.	227.2 ± 17.8 [15]b	222.1 ± 22.3	230.8 ± 20.2	221.5 ± 19.7
DAY 37	MEAN±S.D.	234.2 ± 18.4 [15]b	227.3 ± 22.3	238.4 ± 21.2	227.1 ± 21.3

DAY = DAY OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Access to test diet occurred from day 1 of study until sacrifice.
b. Excludes values for rat 12741, which was moribund sacrificed on day 32 of study.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 3 (PAGE 3): BODY WEIGHTS - SUMMARY - MALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (PPM)a		0	100	1000	10000
RATS TESTED	N	16	16	16	16
INCLUDED IN ANALYSES	N	15b	16	16	16
BODY WEIGHT (G)					
DAY 38	MEAN±S.D.	238.4 ± 19.3	232.2 ± 22.7	242.3 ± 21.0	231.4 ± 20.2
DAY 39	MEAN±S.D.	244.9 ± 18.8	238.3 ± 24.2	249.3 ± 21.3	238.0 ± 20.9
DAY 40	MEAN±S.D.	248.1 ± 19.2	242.1 ± 24.5	252.1 ± 22.2	241.4 ± 19.9
DAY 41	MEAN±S.D.	252.1 ± 20.2	245.6 ± 25.1	256.9 ± 22.4	245.7 ± 20.0
DAY 42	MEAN±S.D.	255.7 ± 20.6	249.2 ± 25.8	261.7 ± 22.5	250.6 ± 21.3
DAY 43	MEAN±S.D.	257.9 ± 20.8	252.6 ± 25.7	264.1 ± 23.5	253.6 ± 21.6
DAY 44	MEAN±S.D.	261.5 ± 21.5	255.6 ± 27.4	266.9 ± 23.7	256.8 ± 22.3
DAY 45	MEAN±S.D.	263.1 ± 21.0	254.4 ± 25.6 [15]c	269.3 ± 24.4	258.3 ± 21.6
DAY 46	MEAN±S.D.	265.5 ± 23.6	257.3 ± 25.9 [15]c	273.1 ± 24.4	262.5 ± 23.0
DAY 47	MEAN±S.D.	269.7 ± 23.7	260.2 ± 25.8 [15]c	276.7 ± 25.1	266.4 ± 23.6
DAY 48	MEAN±S.D.	271.0 ± 23.0	264.3 ± 27.5 [15]c	281.6 ± 25.4	269.3 ± 24.8
DAY 49	MEAN±S.D.	275.8 ± 24.3	266.5 ± 26.6 [15]c	283.0 ± 26.5	273.7 ± 25.1
DAY 50	MEAN±S.D.	278.4 ± 25.6	269.7 ± 26.2 [15]c	286.5 ± 27.3	276.8 ± 25.8
DAY 51	MEAN±S.D.	280.2 ± 27.0	272.8 ± 28.6 [15]c	289.0 ± 27.5	279.2 ± 24.7
DAY 52	MEAN±S.D.	285.4 ± 27.8	276.2 ± 28.4 [15]c	292.6 ± 28.4	283.5 ± 25.6

DAY = DAY OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Access to test diet occurred from day 1 of study until sacrifice.
b. Excludes values for rat 12741, which was moribund sacrificed on day 32 of study.
c. Excludes values for rat 12753, which was moribund sacrificed on day 44 of study.

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TABLE 3 (PAGE 4): BODY WEIGHTS - SUMMARY - MALE RATS

DOSAGE GROUP DOSAGE (PPM)a		I 0	II 100	III 1000	IV 10000
RATS TESTED	N	16	16	16	16
INCLUDED IN ANALYSES	N	15b	15c	16	16
BODY WEIGHT (G)					
DAY 53	MEAN±S.D.	286.6 ± 27.0	278.9 ± 29.4	295.7 ± 29.6	285.6 ± 25.7
DAY 54	MEAN±S.D.	290.4 ± 28.9	283.0 ± 30.0	299.8 ± 30.5	288.7 ± 26.4
DAY 55	MEAN±S.D.	292.5 ± 28.9	285.9 ± 30.1	302.0 ± 31.4	291.9 ± 26.7
DAY 56	MEAN±S.D.	295.6 ± 29.8	288.1 ± 29.8	305.5 ± 31.6	294.8 ± 27.6
DAY 57	MEAN±S.D.	298.7 ± 30.8	291.7 ± 30.5	308.9 ± 31.7	297.7 ± 28.4
DAY 58	MEAN±S.D.	299.2 ± 30.7	291.9 ± 30.3	310.0 ± 33.4	300.2 ± 28.5
DAY 59	MEAN±S.D.	299.8 ± 31.4	292.3 ± 30.9	311.4 ± 33.3	299.9 ± 29.6

DAY = DAY OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Access to test diet occurred from day 1 of study until sacrifice.
b. Excludes values for rat 12741, which was moribund sacrificed on day 32 of study.
c. Excludes values for rat 12753, which was moribund sacrificed on day 44 of study.

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TABLE 4 (PAGE 1): BODY WEIGHT CHANGES - SUMMARY - MALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (PPM)a		0	100	1000	10000
RATS TESTED	N	16	16	16	16
BODY WEIGHT CHANGE (G)					
DAYS 1 - 8	MEAN±S.D.	+30.6 ± 4.4	+27.6 ± 4.8	+29.0 ± 5.3	+27.5 ± 5.2
DAYS 8 - 15	MEAN±S.D.	+41.2 ± 3.6	+41.1 ± 6.1	+42.2 ± 5.2	+39.2 ± 5.4
DAYS 15 - 22	MEAN±S.D.	+45.7 ± 4.6	+43.2 ± 4.1	+45.3 ± 4.4	+43.6 ± 5.1
DAYS 22 - 29	MEAN±S.D.	+37.4 ± 4.1	+37.3 ± 6.2	+39.0 ± 3.6	+37.2 ± 4.8
DAYS 29 - 36	MEAN±S.D.	+34.1 ± 6.0 [15]b	+35.6 ± 5.1	+37.3 ± 4.5	+35.8 ± 4.4
DAYS 36 - 43	MEAN±S.D.	+30.7 ± 5.2 [15]b	+30.5 ± 5.8	+33.2 ± 4.9	+32.2 ± 4.0
DAYS 43 - 50	MEAN±S.D.	+20.6 ± 6.6 [15]b	+20.2 ± 7.3 [15]c	+22.4 ± 5.4	+23.2 ± 5.8
DAYS 50 - 57	MEAN±S.D.	+20.3 ± 7.1 [15]b	+22.0 ± 5.7 [15]c	+22.4 ± 5.8	+20.9 ± 5.3
DAYS 1 - 57	MEAN±S.D.	+259.9± 29.5 [15]b	+254.6± 27.8 [15]c	+270.9± 29.2	+259.5± 26.7

DAYS = DAYS OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Access to test diet occurred from day 1 of study until sacrifice.
b. Excludes rat 12741, which was moribund sacrificed on day 32 of study.
c. Excludes rat 12753, which was moribund sacrificed on day 44 of study.

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TABLE 5 (PAGE 1): ABSOLUTE FEED CONSUMPTION VALUES (G/DAY) - SUMMARY - MALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (PPM)a		0	100	1000	10000
RATS TESTED	N	16	16	16	16
FEED CONSUMPTION (G/DAY)					
DAYS 1 - 8	MEAN±S.D.	9.6 ± 1.0	8.4 ± 1.2**	9.0 ± 1.0	8.6 ± 1.5*
DAYS 8 - 15	MEAN±S.D.	15.6 ± 1.1	14.9 ± 1.7	15.7 ± 1.7	15.0 ± 1.7
DAYS 15 - 22	MEAN±S.D.	19.2 ± 1.2	18.4 ± 2.0	19.0 ± 1.6	18.1 ± 2.0
DAYS 22 - 29	MEAN±S.D.	22.8 ± 1.2	22.3 ± 2.3	22.9 ± 1.9	22.4 ± 2.8
DAYS 29 - 36	MEAN±S.D.	22.5 ± 1.6 [14]b,c	22.1 ± 2.4	23.2 ± 1.6	22.2 ± 2.8
DAYS 36 - 43	MEAN±S.D.	23.5 ± 1.4 [14]b,c	23.4 ± 2.6	24.2 ± 1.7	23.6 ± 3.2
DAYS 43 - 50	MEAN±S.D.	22.0 ± 1.7 [15]c	21.0 ± 2.4 [15]d	22.6 ± 1.9 [15]b	21.9 ± 2.1 [15]b
DAYS 50 - 57	MEAN±S.D.	23.1 ± 2.1 [15]c	22.6 ± 2.3 [14]b,d	24.2 ± 2.7	22.9 ± 2.3 [15]b
DAYS 1 - 57	MEAN±S.D.	19.7 ± 1.1 [15]c	19.1 ± 1.5 [14]b,d	20.1 ± 1.4	19.1 ± 1.7 [15]b

DAYS = DAYS OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Access to test diet occurred from day 1 of study until sacrifice.
b. Excludes values that appeared incorrectly recorded, as well as those that were associated with spillage.
c. Excludes rat 12741, which was moribund sacrificed on day 32 of study.
d. Excludes rat 12753, which was moribund sacrificed on day 44 of study.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

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TABLE 6 (PAGE 1): RELATIVE FEED CONSUMPTION VALUES (G/KG/DAY) - SUMMARY - MALE RATS

DOSAGE GROUP DOSAGE (PPM)a		I 0	II 100	III 1000	IV 10000
RATS TESTED	N	16	16	16	16
FEED CONSUMPTION (G/KG/DAY)					
DAYS 1 - 8	MEAN±S.D.	180.8 ± 15.0	169.9 ± 11.7	177.5 ± 12.1	170.6 ± 16.8
DAYS 8 - 15	MEAN±S.D.	174.0 ± 11.5	176.8 ± 11.1	180.7 ± 16.7	178.5 ± 16.5
DAYS 15 - 22	MEAN±S.D.	144.7 ± 6.8	146.0 ± 7.1	145.8 ± 9.5	144.7 ± 11.6
DAYS 22 - 29	MEAN±S.D.	130.0 ± 6.8	132.7 ± 6.6	131.8 ± 9.8	134.1 ± 11.7
DAYS 29 - 36	MEAN±S.D.	107.1 ± 10.0 [14]b,c	108.5 ± 4.7	109.3 ± 8.7	109.0 ± 8.4
DAYS 36 - 43	MEAN±S.D.	97.1 ± 6.1 [14]b,c	98.0 ± 6.0	97.7 ± 9.9	99.0 ± 8.6
DAYS 43 - 50	MEAN±S.D.	82.6 ± 6.2 [15]c	81.1 ± 5.9 [15]d	81.9 ± 5.8 [15]b	83.8 ± 6.2 [15]b
DAYS 50 - 57	MEAN±S.D.	80.2 ± 5.9 [15]c	80.0 ± 3.6 [14]b,d	81.8 ± 10.4	80.7 ± 5.2 [15]b
DAYS 1 - 57	MEAN±S.D.	108.2 ± 6.5 [15]c	108.6 ± 4.3 [14]b,d	109.3 ± 8.2	108.8 ± 6.8 [15]b

DAYS = DAYS OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Access to test diet occurred from day 1 of study until sacrifice.
b. Excludes values that appeared incorrectly recorded, as well as those that were associated with spillage.
c. Excludes rat 12741, which was moribund sacrificed on day 32 of study.
d. Excludes rat 12753, which was moribund sacrificed on day 44 of study.

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TABLE 7 (PAGE 1): NECROPSY OBSERVATIONS - SUMMARY - MALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (PPM)a		0	100	1000	10000
RATS EXAMINED b	N	16	16	16	16
MORIBUND SACRIFICED	N	1c	1d	0	0
APPEARED NORMAL	N	15	16d	16	16
EYES:					
RIGHT, ANTERIOR CHAMBER OF EYE COLLAPSED	N	1c	0	0	0

a. Access to test diet occurred from day 1 of study until sacrifice.
b. Refer to the individual clinical observations table (Table 14) for external observations confirmed at necropsy.
c. Rat 12741 was moribund sacrificed on day 32 of study.
d. Rat 12753 was moribund sacrificed on day 44 of study.

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TABLE 8 (PAGE 1): TERMINAL BODY WEIGHTS AND ORGAN WEIGHTS - SUMMARY - MALE RATS

DOSAGE GROUP DOSAGE (PPM)a		I 0	II 100	III 1000	IV 10000
RATS TESTED	N	15b	15b	16	16
TERMINAL BODY WEIGHT	MEAN±S.D.	300.8 ± 32.0	294.1 ± 31.6	312.5 ± 33.5	301.2 ± 31.3
EPIDIDYMIS LEFT	MEAN±S.D.	0.5617 ±0.0535	0.5280 ±0.0658	0.5297 ±0.0461	0.5636 ±0.0890
CAUDA EPIDIDYMIS LEFT	MEAN±S.D.	0.2068 ±0.0297	0.2034 ±0.0408	0.1997 ±0.0258	0.2094 ±0.0405
TESTIS LEFT	MEAN±S.D.	1.6290 ±0.1536	1.6320 ±0.1474	1.6524 ±0.1590	1.6352 ±0.1197
L. TESTIS MINUS TUNICA ALBUGINEA	MEAN±S.D.	1.5031 ±0.1739	1.4987 ±0.1380	1.5217 ±0.1393	1.4950 ±0.1259
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	0.7893 ±0.1453	0.7923 ±0.1233	0.8296 ±0.1532	0.8138 ±0.1861
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	0.4574 ±0.0991	0.4556 ±0.0759	0.4663 ±0.0811	0.4589 ±0.0849
EPIDIDYMIS RIGHT	MEAN±S.D.	0.5324 ±0.0511	0.5412 ±0.0657	0.5229 ±0.0559	0.5982 ±0.2408
TESTIS RIGHT	MEAN±S.D.	1.6024 ±0.1186	1.6461 ±0.1524	1.6301 ±0.1294	1.6276 ±0.1277
PROSTATE VENTRAL	MEAN±S.D.	0.3206 ±0.0770 [14]c	0.2956 ±0.0487	0.3198 ±0.0616	0.3273 ±0.0805
PITUITARY	MEAN±S.D.	0.008 ± 0.003 [14]c	0.008 ± 0.002	0.009 ± 0.002	0.009 ± 0.003
LIVER	MEAN±S.D.	11.93 ± 1.56	11.62 ± 1.61	12.30 ± 1.58	12.22 ± 1.88
ADRENALS PAIRED	MEAN±S.D.	0.064 ± 0.010	0.069 ± 0.020	0.064 ± 0.011	0.070 ± 0.017
THYROID	MEAN±S.D.	0.024 ± 0.010	0.022 ± 0.005	0.024 ± 0.007	0.027 ± 0.011
PROSTATE DORSAL	MEAN±S.D.	0.3413 ±0.0879	0.3269 ±0.0571	0.3781 ±0.1190	0.2894 ±0.1091

ALL WEIGHTS WERE RECORDED IN GRAMS (G).
[ ] = NUMBER OF VALUES AVERAGED
a. Access to test diet occurred from day 1 of study until sacrifice.
b. Excludes values for rats that were moribund sacrificed.
c. Excludes values that were not recorded.

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TABLE 9 (PAGE 1): RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - SUMMARY - MALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (PPM)a		0	100	1000	10000
RATS TESTED	N	15b	15b	16	16
TERMINAL BODY WEIGHT	MEAN±S.D.	300.8 ± 32.0	294.1 ± 31.6	312.5 ± 33.5	301.2 ± 31.3
EPIDIDYMIS LEFT	MEAN±S.D.	0.187 ± 0.024	0.179 ± 0.016	0.171 ± 0.018	0.186 ± 0.019
CAUDA EPIDIDYMIS LEFT	MEAN±S.D.	0.069 ± 0.011	0.069 ± 0.012	0.063 ± 0.009	0.069 ± 0.011
TESTIS LEFT	MEAN±S.D.	0.545 ± 0.054	0.559 ± 0.046	0.529 ± 0.042	0.546 ± 0.045
L. TESTIS MINUS TUNICA ALBUGINEA	MEAN±S.D.	0.501 ± 0.054	0.513 ± 0.046	0.487 ± 0.040	0.497 ± 0.043
SEMINAL VESICLES WITH FLUID	MEAN±S.D.	0.265 ± 0.059	0.270 ± 0.038	0.267 ± 0.056	0.271 ± 0.062
SEMINAL VESICLES WITHOUT FLUID	MEAN±S.D.	0.154 ± 0.040	0.155 ± 0.022	0.149 ± 0.028	0.151 ± 0.030
EPIDIDYMIS RIGHT	MEAN±S.D.	0.178 ± 0.028	0.185 ± 0.022	0.168 ± 0.018	0.201 ± 0.094
TESTIS RIGHT	MEAN±S.D.	0.535 ± 0.051	0.560 ± 0.054	0.523 ± 0.038	0.542 ± 0.051
PROSTATE VENTRAL	MEAN±S.D.	0.106 ± 0.023 [14]c	0.101 ± 0.023	0.103 ± 0.022	0.108 ± 0.024
PITUITARY d	MEAN±S.D.	2.841 ± 0.983 [14]c	2.877 ± 0.911	2.798 ± 0.783	2.953 ± 1.056
LIVER	MEAN±S.D.	3.963 ± 0.277	3.947 ± 0.272	3.932 ± 0.254	4.043 ± 0.288
ADRENALS PAIRED	MEAN±S.D.	0.021 ± 0.004	0.023 ± 0.008	0.019 ± 0.004	0.022 ± 0.006
THYROID d	MEAN±S.D.	8.093 ± 3.196	7.378 ± 1.491	7.550 ± 2.128	9.179 ± 3.990
PROSTATE DORSAL	MEAN±S.D.	0.112 ± 0.026	0.111 ± 0.022	0.120 ± 0.036	0.094 ± 0.036

ALL WEIGHTS WERE RECORDED IN GRAMS (G). RATIOS (%) = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.
[ ] = NUMBER OF VALUES AVERAGED
a. Access to test diet occurred from day 1 of study until sacrifice.
b. Excludes values for rats that were moribund sacrificed.
c. Excludes values that were not recorded.
d. Value was multiplied by 1000.

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TABLE 10 (PAGE 1): SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - SUMMARY - MALE RATS

DOSAGE GROUP		I			II			III			IV		
DOSAGE (PPM)a		0			100			1000			10000		
RATS TESTED	N	15b			15b			16			16		
VAS DEFERENS SPERM MOTILITY													
NUMBER MOTILE	MEAN±S.D.	306.8	±	75.5	345.3	±	138.4	275.2	±	69.0	298.2	±	97.8
MOTILE PERCENT	MEAN±S.D.	94.4	±	3.1	92.2	±	7.1	90.3	±	7.9	91.4	±	4.5
STATIC COUNT (NONMOTILE)	MEAN±S.D.	19.3	±	15.3	32.7	±	45.0	28.4	±	21.5	27.6	±	15.7
TOTAL COUNT c	MEAN±S.D.	326.1	±	86.7	378.0	±	156.9	303.6	±	67.2	325.9	±	104.6
CAUDA EPIDIDYMAL SPERM COUNT													
SPERM COUNT d	MEAN±S.D.	49.7	±	25.0	78.3	±	75.8	56.4	±	28.1	49.4	±	16.8
SPERM DENSITY e	MEAN±S.D.	701.86	±	385.77	1079.49	±	773.23	794.92	±	322.96	710.64	±	293.34

a. Access to test diet occurred on days 1 thorough 56 of study.
b. Excludes values for rats that were moribund sacrificed.
c. Sum of number motile and static count. Groups of five fields were evaluated until a sperm count of at least 200 was achieved or 20 fields were evaluated.
d. Sperm count used in the calculation of sperm density. Ten fields were evaluated.
e. The sperm density was calculated by dividing the sperm count by the volume in the image area (34.3×10^{-6} mL), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) was multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table 18 for the weight of the left cauda epididymis) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

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TABLE 10 (PAGE 2): SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - SUMMARY - MALE RATS

DOSAGE GROUP		I			II			III			IV		
DOSAGE (PPM)a		0			100			1000			10000		
RATS TESTED	N	15b			15b			16			16		
TESTICULAR SPERMATID COUNT													
SPERMATID COUNT c	MEAN±S.D.	77.2	±	23.8	66.3	±	28.8	65.2	±	26.4	68.6	±	32.1
SPERMATID DENSITY d	MEAN±S.D.	148.86	±	42.68	127.44	±	52.29	126.79	±	56.36	130.31	±	55.44
DAILY SPERM PRODUCTON e	MEAN±S.D.	36.82	±	11.38	31.81	±	13.80	31.09	±	12.53	32.83	±	15.37

a. Access to test diet occurred on days 1 thorough 56 of study.
b. Excludes values for rats that were moribund sacrificed.
c. Spermatid count used in the calculation of spermatid density. Ten fields were evaluated.
d. The spermatid density was calculated by dividing the spermatid count by the volume in the image area (34.3×10^{-6} mL), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the spermatid concentration. The calculated spermatid concentration value (rounded to 1 decimal place) was multiplied by 50 (volume) and divided by the weight of the left testis minus tunica albuginea (see Table 18 for the weight of the left testis minus tunica albuginea) to obtain the spermatid density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.
e. The daily sperm production was calculated by dividing the spermatid count by the volume in the image area (34.3×10^{-6} mL), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the spermatid concentration. The calculated spermatid concentration value (rounded to 1 decimal place) was multiplied by 50 (volume) and divided by 6.1 days (which is the transit time for spermatids).

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TABLE 11 (PAGE 1): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - SUMMARY - MALE RATS

DOSAGE GROUP DOSAGE (PPM)a		I 0	II 100	III 1000	IV 10000
RATS EXAMINED	N	15	15	16	16
NORMAL	MEAN±S.D.	197.5 ± 2.6	196.4 ± 2.7	197.0 ± 2.0	196.4 ± 2.5
PER-CENT ABNORMAL	MEAN±S.D.	1.3 ± 1.3	1.8 ± 1.3	1.5 ± 1.0	1.8 ± 1.2
DETACHED HEAD	MEAN±S.D.	1.3 ± 2.0	2.0 ± 1.6	1.5 ± 1.3	2.3 ± 2.2
NO HEAD	MEAN±S.D.	0.8 ± 1.2	1.5 ± 1.5	1.2 ± 1.5	1.1 ± 1.1
BROKEN FLAGELLUM	MEAN±S.D.	0.3 ± 0.6	0.1 ± 0.2	0.1 ± 0.3	0.2 ± 0.5
PIN HEAD	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
TWO HEADS & TAILS	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
SHORT SPERM HEAD	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
BANANA	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
COILED FLAGELLUM	MEAN±S.D.	0.1 ± 0.2	0.1 ± 0.2	0.1 ± 0.3	0.0 ± 0.0
BENT FLAGELLUM	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
BENT FLAGELLUM TIP	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
OTHER	MEAN±S.D.	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

a. Access to test diet occurred from day 1 of study until sacrifice.

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TABLE 12 (PAGE 1): TESTOSTERONE LEVELS (NG/ML) - SUMMARY - MALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (PPM)a		0	100	1000	10000
RATS TESTED		16	16	16	16
TESTOSTERONE LEVELS (ng/mL)					
WEEK 3	MEAN±S.D.	0.503 ± 0.411	0.351 ± 0.088	0.325 ± 0.101*	0.257 ± 0.077**
WEEK 5	MEAN±S.D.	1.417 ± 1.141	1.249 ± 0.840	1.166 ± 0.966	0.922 ± 0.528
WEEK 7	MEAN±S.D.	1.472 ± 0.659 [15]b	2.150 ± 1.546	1.264 ± 0.495	2.174 ± 0.981
WEEK 9	MEAN±S.D.	1.085 ± 0.430 [15]b	1.485 ± 0.767 [15]b	1.031 ± 0.575	1.860 ± 0.968**

WEEK = WEEK OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Access to test diet occurred from day 1 of study until sacrifice.
b. Excludes values for rats that were moribund sacrificed.
* Significantly different from the control group value ($p \leq 0.05$).
** Significantly different from the control group value ($p \leq 0.01$).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 13 (PAGE 1): FOLLICLE STIMULATING HORMONE LEVELS (NG/ML) - SUMMARY - MALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (PPM)a		0	100	1000	10000
RATS TESTED		16	16	16	16
FOLLICLE STIMULATING HORMONE LEVELS (ng/mL)					
WEEK 3	MEAN±S.D.	103.115 ± 26.565	99.899 ± 17.718	104.934 ± 23.575	98.500 ± 20.048
WEEK 5	MEAN±S.D.	100.206 ± 30.102	89.975 ± 24.038	108.343 ± 30.164	95.629 ± 27.155
WEEK 7	MEAN±S.D.	137.030 ± 36.272 [15]b	130.028 ± 48.795	139.609 ± 57.097	111.378 ± 33.202
WEEK 9	MEAN±S.D.	129.782 ± 58.506 [13]b,c	146.805 ± 79.988 [15]b	130.605 ± 68.414	198.700 ± 82.781*

WEEK = WEEK OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Access to test diet occurred from day 1 of study until sacrifice.
b. Excludes values for rats that were moribund sacrificed.
c. Excludes values that were beyond the validated range.
* Significantly different from the control group value (p≤0.05).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 14 (PAGE 1): LUTENIZING HORMONE LEVELS (NG/ML) - SUMMARY - MALE RATS

DOSAGE GROUP		I	II	III	IV
DOSAGE (PPM)a		0	100	1000	10000
RATS TESTED		16	16	16	16
LUTENIZING HORMONE LEVELS (ng/mL)					
WEEK 3	MEAN±S.D.	4.253 ± 1.197	3.997 ± 1.171	3.916 ± 1.523	3.482 ± 1.040
WEEK 5	MEAN±S.D.	5.929 ± 1.465	3.825 ± 1.214**	5.115 ± 1.828	4.131 ± 1.319**
WEEK 7	MEAN±S.D.	5.465 ± 1.889 [15]b	4.377 ± 0.834	4.909 ± 1.386	5.319 ± 2.152
WEEK 9	MEAN±S.D.	4.060 ± 0.883 [15]b	3.554 ± 1.175 [15]b	3.781 ± 0.753	3.690 ± 0.716

WEEK = WEEK OF STUDY
[ ] = NUMBER OF VALUES AVERAGED
a. Access to test diet occurred from day 1 of study until sacrifice.
b. Excludes values for rats that were moribund sacrificed.
** Significantly different from the control group value (p≤0.01).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 15 (PAGE 1): CLINICAL OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP I	CARRIER CONTROL	0 PPM
RAT #		DESCRIPTION
12737		NO ADVERSE FINDINGS
12738	DS(6- 59)	INCISOR(S): MISALIGNED a
	DS(45- 47)	CHROMORHINORRHEA
	DS(59)	CHROMODACRYORRHEA a
12739	DS(53- 59)	BACK: SCAB (1.0 CM X 0.5 CM)a
	DS(59)	CHROMODACRYORRHEA a
12740	DS(46- 47)	CHROMORHINORRHEA
	DS(46- 50)	CHROMODACRYORRHEA
	DS(49- 50)	CHROMORHINORRHEA
12741	DS(30- 32)	RIGHT EYE: TRAUMATIZED CORNEA a
	DS(30- 32)	RIGHT EYE: EXOPHTHALMOS a
	DS(30- 32)	RIGHT EYE: DRIED CORNEA
	DS(30- 32)	RIGHT EYE: TRAUMATIZED CONJUCTIVA
	DS(31- 32)	RIGHT EYE: CORNEAL DESSICATION
	DS(31- 32)	RIGHT EYE: CORNEAL ULCERATION
	DS(31- 32)	HEAD: SWOLLEN a
	DS(32)	MORIBUND SACRIFICED
12742	DS(48)	CHROMORHINORRHEA
12743	DS(10- 11)	URINE-STAINED ABDOMINAL FUR
	DS(18- 21)	INCISOR(S): MISSING/BROKEN
	DS(46- 47)	BACK: ABRASION (1.2 CM X 0.5 CM)
	DS(48- 59)	BACK: SCAB (DID NOT EXCEED 2.0 CM X 1.0 CM)
	DS(60)	BACK: ULCERATION (2.0 CM X 1.0 CM)a
12744		NO ADVERSE FINDINGS
12745	DS(16- 60)	LEFT EYE: ENLARGED a
	DS(44- 60)	LEFT EYE: CORNEAL OPACITY a
12746	DS(9- 60)	RIGHT EYE: ENLARGED a
	DS(33- 34)	CHROMODACRYORRHEA
	DS(45- 55)	CHROMODACRYORRHEA
12747	DS(18- 31)	HEAD: BELOW RIGHT EYE, SCAB (DID NOT EXCEED 0.5 CM X 0.1 CM)
	DS(32- 34)	NECK: SCAB (1.5 CM X 0.8 CM)
	DS(35)	NOSE: SCAB (0.3 CM IN DIAMETER)
	DS(47)	CHROMORHINORRHEA
	DS(49- 50)	CHROMORHINORRHEA
12748	DS(47- 48)	CHROMORHINORRHEA
	DS(48)	CHROMODACRYORRHEA
12749	DS(8- 10)	INCISOR(S): MISSING/BROKEN
12750		NO ADVERSE FINDINGS
12751		NO ADVERSE FINDINGS
12752		NO ADVERSE FINDINGS

DS = DAY OF STUDY
a. Observation confirmed at necropsy.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 15 (PAGE 2): CLINICAL OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP II	LOW DOSAGE	100 PPM
RAT #	DESCRIPTION	
12753	DS(33- 41)	LEFT AXILLA: SCAB (0.5 CM X 0.2 CM)
	DS(44)	VOCALIZATION
	DS(44)	RIGHT EYE: EXOPHTHALMOS a
	DS(44)	RIGHT EYE: RED PERIORBITAL SUBSTANCE a
	DS(44)	RIGHT EYE: UNREACTIVE PUPIL
	DS(44)	RIGHT EYE: PERIORBITAL DISCOLORATION a
	DS(44)	HEAD: SWOLLEN a
	DS(44)	HEAD TILT
	DS(44)	MORIBUND SACRIFICED
12754		NO ADVERSE FINDINGS
12755	DS(47)	CHROMORHINORRHEA
	DS(47- 52)	CHROMODACRYORRHEA
	DS(50)	CHROMORHINORRHEA
12756	DS(32- 33)	MOUTH: ULCERATION (0.2 CM X 0.2 CM)
	DS(34)	MOUTH: SCAB (0.1 CM IN DIAMETER)
12757		NO ADVERSE FINDINGS
12758	DS(26)	CHROMORHINORRHEA
12759	DS(33- 60)	TAIL BENT a
	DS(45- 47)	CHROMORHINORRHEA
	DS(47- 53)	CHROMODACRYORRHEA
	DS(49- 53)	CHROMORHINORRHEA
12760	DS(45- 50)	CHROMORHINORRHEA
	DS(47)	CHROMODACRYORRHEA
	DS(49- 52)	CHROMODACRYORRHEA
	DS(52)	CHROMORHINORRHEA
12761	DS(34- 60)	HEAD TILT
	DS(46- 50)	CHROMORHINORRHEA
	DS(47- 53)	CHROMODACRYORRHEA
	DS(52- 53)	CHROMORHINORRHEA
	DS(55- 56)	CHROMORHINORRHEA
	DS(59)	CHROMORHINORRHEA

DS = DAY OF STUDY
a. Observation confirmed at necropsy.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 15 (PAGE 3): CLINICAL OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP II	LOW DOSAGE	100 PPM

RAT #		DESCRIPTION
12762	DS(32- 45)	LOCALIZED ALOPECIA: LIMB(S)
12763		NO ADVERSE FINDINGS
12764	DS(30- 49)	NECK: SCAB (0.5 CM X 1.0 CM)
	DS(32- 40)	MOUTH: SCAB (0.5 CM X 0.3 CM)
	DS(35- 40)	NOSE: SCAB (0.5 CM X 0.2 CM)
	DS(35- 40)	HEAD: SCAB (0.5 CM X 0.3 CM)
	DS(41)	SPARSE COAT
	DS(41- 44)	LOCALIZED ALOPECIA: HEAD
12765		NO ADVERSE FINDINGS
12766	DS(8- 9)	RIGHT EYE: CORNEAL OPACITY
	DS(8- 11)	ENLARGED EYE
	DS(16- 19)	CHROMORHINORRHEA
	DS(44)	CHROMORHINORRHEA
12767		NO ADVERSE FINDINGS
12768	DS(34- 37)	NOSE: SCAB (0.2 CM X 0.1 CM)
	DS(38- 39)	NECK: SCAB (0.4 CM IN DIAMETER)

DS = DAY OF STUDY

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 15 (PAGE 4): CLINICAL OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP III		MIDDLE DOSAGE	1000 PPM
RAT #		DESCRIPTION	
12769		NO ADVERSE FINDINGS	
12770	DS(45)	CHROMORHINORRHEA	
12771		NO ADVERSE FINDINGS	
12772		NO ADVERSE FINDINGS	
12773	DS(44- 45)	RIGHT EYE: TRAUMATIZED CORNEA	
	DS(47)	CHROMORHINORRHEA	
	DS(49)	CHROMORHINORRHEA	
12774		NO ADVERSE FINDINGS	
12775	DS(45- 47)	CHROMODACRYORRHEA	
	DS(45- 49)	BACK: SCAB (1.5 CM X 1.0 CM)	
12776	DS(38- 39)	CHROMORHINORRHEA	
	DS(49)	CHROMORHINORRHEA	
12777		NO ADVERSE FINDINGS	
12778	DS(45)	CHROMODACRYORRHEA	
12779	DS(57- 59)	NECK: ABRASION (0.3 CM IN DIAMETER)a	
	DS(57- 60)	LOCALIZED ALOPECIA: NECK	
	DS(60)	NECK: SCAB (0.3 CM IN DIAMETER)a	
12780		NO ADVERSE FINDINGS	
12781		NO ADVERSE FINDINGS	
12782		NO ADVERSE FINDINGS	
12783		NO ADVERSE FINDINGS	
12784		NO ADVERSE FINDINGS	

DS = DAY OF STUDY
a. Observation confirmed at necropsy.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 15 (PAGE 5): CLINICAL OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP IV	HIGH DOSAGE	10000 PPM
RAT #	DESCRIPTION	
12785	DS(33- 39)	CHROMODACRYORRHEA
	DS(34- 39)	CHROMORHINORRHEA
	DS(35- 40)	SPARSE COAT
	DS(38)	LACRIMATION
	DS(45)	CHROMODACRYORRHEA
	DS(48)	CHROMORHINORRHEA
12786		NO ADVERSE FINDINGS
12787		NO ADVERSE FINDINGS
12788	DS(9- 10)	URINE-STAINED ABDOMINAL FUR
12789		NO ADVERSE FINDINGS
12790	DS(40)	EXCESS SALIVATION
12791	DS(17- 23)	CHROMORHINORRHEA
	DS(41)	CHROMORHINORRHEA
	DS(45- 49)	CHROMODACRYORRHEA
	DS(48)	CHROMORHINORRHEA
12792	DS(10)	URINE-STAINED ABDOMINAL FUR
12793	DS(9- 10)	URINE-STAINED ABDOMINAL FUR
12794		NO ADVERSE FINDINGS
12795	DS(47)	CHROMORHINORRHEA
12796	DS(26- 61)	INCISOR(S): MISALIGNED a
	DS(30- 43)	NECK: SCAB (1.5 CM X 0.4 CM)
	DS(37- 61)	INCISOR(S): MISSING/BROKEN a
	DS(45- 61)	LOCALIZED ALOPECIA: LIMB(S)a
	DO(44- 61)	LOCALIZED ALOPECIA: NECK a
	DS(56- 61)	NOSE: SCAB (1.0 CM X 0.5 CM)a
	DS(61)	NECK: MULTIPLE, SCABS (0.2 CM IN DIAMETER)a
	DS(61)	SPARSE COAT a
12797	DS(32- 34)	CHROMODACRYORRHEA
12798		NO ADVERSE FINDINGS
12799		NO ADVERSE FINDINGS
12800		NO ADVERSE FINDINGS

DS = DAY OF STUDY
a. Observation confirmed at necropsy.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 1): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP I				CARRIER CONTROL				0 PPM				
DAY	1	2	3	4	5	6	7	8	9	10	11	12	13
12737	42.8	46.9	51.9	55.9	61.8	66.2	71.3	76.7	81.8	88.1	95.2	99.6	106.0
12738	42.7	45.1	48.7	55.1	57.6	63.8	68.4	69.1	73.7	78.4	84.8	90.9	98.1
12739	34.2	32.6	36.2	39.5	44.9	50.4	55.0	59.9	63.9	70.6	76.6	82.4	86.6
12740	42.8	42.5	46.1	51.7	58.4	64.4	69.5	73.1	77.9	84.0	90.7	96.0	102.2
12741	39.1	42.7	44.2	49.1	56.2	61.0	66.0	71.5	76.8	84.2	91.0	96.4	102.6
12742	41.4	45.9	46.8	51.5	57.2	60.9	65.8	72.6	75.4	80.6	87.2	94.4	100.0
12743	31.7	36.4	39.4	43.5	48.1	52.9	56.4	59.7	64.6	71.5	76.8	82.1	88.3
12744	35.2	38.9	43.1	49.5	54.4	60.7	66.1	68.2	74.1	81.5	86.4	92.3	101.0
12745	38.8	44.2	48.5	56.2	61.3	65.5	71.6	76.9	81.5	88.1	96.0	100.9	107.3
12746	35.7	41.2	44.1	50.7	56.6	62.9	67.8	72.9	75.1	81.4	87.2	93.7	97.7
12747	38.5	40.5	44.2	50.5	55.6	59.7	63.2	66.0	69.0	75.4	83.7	89.5	95.3
12748	41.9	44.6	50.0	56.5	60.7	66.5	70.9	74.1	78.7	86.3	93.9	99.6	105.8
12749	37.4	35.0	38.3	44.2	46.7	49.8	54.9	59.9	64.0	70.2	75.5	81.0	86.6
12750	38.8	40.3	46.6	53.2	58.9	63.0	68.2	74.8	80.3	87.2	93.1	98.4	105.1
12751	42.4	40.6	44.2	48.1	54.6	60.8	63.9	69.1	75.5	80.6	86.6	93.6	98.9
12752	38.6	38.0	42.5	48.3	51.4	58.4	61.6	67.5	72.8	77.6	84.5	91.5	97.0

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 2): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP I					CARRIER CONTROL				0 PPM			
	DAY 14	15	16	17	18	19	20	21	22	23	24	25	26
12737	112.7	118.4	121.0	127.4	135.4	140.8	148.0	153.9	161.5	165.9	172.8	176.6	184.2
12738	103.1	108.9	114.1	124.6	130.7	136.2	143.8	151.5	156.0	163.5	171.6	178.3	182.0
12739	91.0	96.2	98.7	107.4	114.1	121.9	124.3	129.7	137.9	147.0	146.9	155.5	158.4
12740	108.3	114.9	119.7	126.0	132.7	139.6	149.0	154.2	160.6	167.5	173.9	176.8	183.4
12741	109.8	118.4	119.8	128.0	139.6	146.0	150.4	155.6	165.7	170.0	173.1	180.0	188.3
12742	105.0	111.7	116.6	124.2	130.2	137.4	144.5	148.5	157.9	161.9	167.3	168.2	179.6
12743	91.8	99.1	100.5	109.5	116.2	121.2	122.9	128.8	136.3	142.4	146.7	150.4	157.2
12744	106.7	113.8	118.5	125.9	135.9	139.2	148.3	153.6	161.2	168.6	173.2	178.0	183.1
12745	116.1	122.2	123.9	136.4	142.7	147.2	154.7	160.7	170.3	175.5	180.6	187.9	195.4
12746	103.5	108.8	113.3	120.7	127.8	133.0	135.7	143.2	150.7	154.9	164.1	169.9	170.9
12747	101.9	108.7	109.0	119.1	124.8	128.4	136.9	143.6	151.1	152.5	159.7	165.0	167.5
12748	112.9	119.8	126.2	134.6	143.3	147.2	156.8	163.0	167.4	175.1	183.4	192.0	193.8
12749	90.8	95.4	100.8	109.2	112.7	119.8	123.2	130.4	135.7	140.4	145.7	150.0	154.4
12750	112.7	117.6	123.7	133.9	141.0	146.2	153.8	163.5	172.2	173.7	182.8	193.2	194.9
12751	106.9	110.4	118.2	125.9	134.2	141.3	148.2	157.6	164.9	168.9	173.5	178.9	185.3
12752	104.5	107.5	112.2	120.8	126.9	132.5	137.7	148.6	153.2	160.7	164.6	170.4	177.5

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 3): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP I					CARRIER CONTROL				0 PPM			
	DAY 27	28	29	30	31	32	33	34	35	36	37	38	39
12737	191.8	194.3	199.9	204.2	209.5	213.3	220.7	227.5	233.9	232.4	239.0	243.4	250.2
12738	190.9	196.4	196.7	198.7	203.9	208.6	211.5	219.9	223.5	224.5	236.2	240.2	251.3
12739	163.7	165.5	172.9	178.6	180.3	188.4	192.9	201.9	204.5	203.9	212.3	213.4	222.5
12740	188.9	194.1	199.5	204.4	208.0	212.9	219.7	225.7	237.3	235.2	242.1	248.0	253.2
12741	191.5	196.0	204.6	204.4	195.0	202.2	MORIBUND SACRIFICED ON DAY 32 OF STUDY						
12742	178.6	184.4	188.3	188.9	191.5	202.1	201.5	205.5	212.8	213.1	217.7	222.1	226.1
12743	161.5	164.3	170.5	175.9	180.1	183.4	187.4	191.2	201.2	202.1	207.6	212.3	217.2
12744	191.6	195.2	204.0	207.3	213.2	222.2	227.7	234.0	240.8	240.9	255.2	253.9	263.6
12745	198.6	202.9	209.8	215.0	217.9	226.0	227.0	231.5	240.0	240.1	249.3	251.4	260.9
12746	178.1	183.0	188.4	189.4	192.5	194.0	200.6	207.5	210.6	213.5	220.4	222.5	228.8
12747	177.2	179.4	185.1	188.1	194.5	203.4	203.1	211.0	221.0	220.4	228.8	233.1	240.6
12748	205.0	209.5	213.1	219.3	226.0	229.2	238.8	245.4	249.9	255.8	264.0	267.3	274.8
12749	159.2	163.4	166.2	172.2	175.6	180.7	188.4	190.9	196.5	202.1	205.6	207.9	216.2
12750	202.5	208.1	210.9	222.8	226.8	232.1	237.6	248.8	246.6	256.2	257.5	269.1	268.7
12751	191.1	194.5	200.5	203.8	211.7	213.4	221.8	225.0	229.5	236.7	240.9	247.3	251.4
12752	182.4	186.2	190.3	197.0	202.5	209.8	213.5	220.1	224.8	230.7	235.7	243.7	247.5

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 4): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP I					CARRIER CONTROL				0 PPM			
	DAY 40	41	42	43	44	45	46	47	48	49	50	51	52
12737	253.7	257.6	258.8	261.5	265.8	267.2	269.0	274.8	276.6	284.4	285.2	287.6	295.7
12738	251.0	251.3	257.3	260.3	264.6	264.0	262.2	270.4	273.4	275.7	279.0	285.0	292.5
12739	226.8	228.4	232.0	232.6	241.1	240.0	239.2	248.7	250.3	255.5	254.7	252.9	260.8
12740	260.3	269.5	270.8	271.0	274.5	275.1	281.1	283.8	286.4	287.9	289.5	295.5	297.9
12741	MORIBUND SACRIFICED ON DAY 32 OF STUDY												
12742	228.9	232.1	237.4	239.4	241.9	243.9	241.7	246.3	246.8	254.2	256.0	255.1	260.5
12743	221.4	226.2	226.5	231.4	232.7	235.6	236.2	237.5	238.7	240.6	242.9	244.2	248.3
12744	267.0	273.5	276.2	277.7	287.2	289.5	293.2	296.9	300.0	307.4	313.1	314.0	316.2
12745	260.5	263.1	267.5	269.4	276.9	276.5	278.7	280.3	283.6	287.3	288.2	290.3	300.2
12746	231.7	234.5	237.7	237.8	243.4	243.9	244.5	249.3	251.7	256.3	257.3	257.9	262.7
12747	244.7	251.9	254.5	259.8	265.3	263.8	269.1	274.2	275.7	282.3	284.2	285.2	291.7
12748	275.7	282.1	287.6	288.5	291.2	293.8	295.0	301.0	302.0	302.8	309.8	310.8	317.9
12749	217.4	217.9	221.8	221.3	219.6	225.7	225.1	224.3	226.1	226.2	227.6	224.8	224.4
12750	278.1	279.4	283.9	286.7	288.6	288.9	302.9	303.2	293.8	308.4	311.2	313.6	319.5
12751	256.6	260.5	263.3	268.3	266.1	270.6	273.1	281.0	281.4	286.0	290.1	293.6	296.5
12752	248.1	253.7	260.4	262.4	263.1	267.4	270.9	274.0	277.8	282.0	287.8	291.8	295.8

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 5): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP I				CARRIER CONTROL			0 PPM	
	DAY 53	54	55	56	57	58	59	60	61
12737	297.1	297.4	304.3	308.4	309.6	309.7	312.5		
12738	292.9	294.8	298.6	300.4	307.3	307.3	304.3		
12739	261.6	266.0	268.8	267.3	267.5	271.6	268.5		
12740	300.0	308.4	307.3	314.4	319.0	318.1	314.1		
12741	MORIBUND SACRIFICED ON DAY 32 OF STUDY								
12742	259.2	261.7	263.6	272.0	273.8	271.5	270.3		
12743	251.5	253.1	255.4	257.4	259.3	262.5	261.5	264.4	
12744	319.2	327.4	328.4	333.8	335.7	343.5	339.9	343.2	
12745	297.8	298.5	300.0	303.6	309.7	308.1	309.3	314.4	
12746	266.7	268.0	269.6	270.4	274.4	271.0	270.6	267.5	
12747	293.3	301.7	305.6	307.8	312.9	314.1	313.0	315.5	
12748	319.2	321.0	320.9	326.0	331.6	332.1	333.9	333.8	339.5
12749	229.2	229.9	228.4	229.3	230.0	232.2	234.8	236.5	
12750	320.3	332.2	328.2	331.0	335.0	330.3	342.1	339.8	
12751	295.6	297.0	304.3	304.2	305.1	306.6	310.6	310.0	
12752	295.1	298.2	303.6	308.0	310.3	309.7	311.3	312.0	

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 6): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP II				LOW DOSAGE				100 PPM				
DAY	1	2	3	4	5	6	7	8	9	10	11	12	13
12753	41.6	44.8	48.9	53.5	57.9	62.4	68.2	73.4	77.0	84.3	91.7	98.1	104.1
12754	44.1	48.1	52.7	57.6	62.5	69.5	77.3	81.5	87.2	93.3	103.0	110.5	117.7
12755	31.9	33.3	34.0	36.7	40.8	47.1	52.3	55.7	58.6	63.5	67.7	73.7	78.7
12756	37.0	37.6	39.9	44.5	51.2	56.2	60.2	63.0	68.3	72.0	77.7	81.1	89.1
12757	39.5	44.7	44.0	47.2	53.1	57.6	61.6	69.3	73.5	76.9	84.0	90.7	96.0
12758	42.6	47.0	52.3	57.4	62.0	67.2	69.0	74.5	80.0	86.8	92.6	103.7	107.7
12759	37.9	36.6	34.1	43.4	47.1	51.9	56.5	61.8	67.7	73.1	79.2	86.1	90.6
12760	33.1	36.6	40.4	46.0	50.1	54.4	60.0	64.5	70.6	77.0	81.8	88.6	97.8
12761	28.3	30.3	32.8	35.5	40.9	44.8	49.2	53.2	56.3	62.1	67.5	71.2	77.7
12762	33.4	36.4	38.7	43.9	48.4	51.9	55.6	61.8	64.7	70.3	75.1	81.9	85.5
12763	41.0	37.2	33.5	43.5	48.0	52.3	54.7	58.5	62.7	70.9	77.1	84.0	87.6
12764	39.3	41.3	44.7	51.4	56.9	61.7	68.2	71.1	75.6	84.2	90.4	95.9	102.1
12765	37.9	34.2	41.8	48.6	53.8	58.2	61.8	67.9	74.0	78.4	85.8	90.8	97.7
12766	34.8	34.3	37.6	41.4	46.5	49.8	53.3	58.5	64.6	68.4	72.1	78.8	83.3
12767	39.5	36.5	38.2	43.2	47.2	52.2	58.2	64.5	69.0	73.0	79.1	85.0	90.3
12768	36.1	34.4	37.1	40.8	45.6	49.2	54.2	59.8	66.5	69.2	74.6	79.4	84.8

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 7): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP II					LOW DOSAGE				100 PPM			
	DAY 14	15	16	17	18	19	20	21	22	23	24	25	26
12753	111.9	122.3	125.4	130.3	139.7	148.4	154.2	160.1	169.5	177.9	181.5	186.9	198.1
12754	126.5	133.6	138.3	145.2	156.2	157.6	167.2	175.8	182.2	184.7	197.0	197.8	203.9
12755	83.4	88.3	92.2	97.5	107.2	111.4	116.6	122.3	129.1	133.9	138.9	143.4	148.6
12756	93.9	100.6	101.8	108.2	119.0	122.0	132.5	137.4	143.0	147.4	158.8	162.5	164.7
12757	102.2	108.6	112.4	120.8	126.4	134.3	140.4	145.2	154.8	157.0	161.8	169.8	172.1
12758	112.4	118.8	118.5	125.4	134.2	139.3	144.9	149.9	156.1	164.4	168.1	173.5	180.6
12759	96.4	104.3	107.0	112.7	120.3	126.2	133.0	140.4	145.4	156.6	163.5	165.5	171.7
12760	103.4	111.5	113.4	122.0	129.7	134.6	141.7	146.3	154.9	157.0	165.7	170.3	173.6
12761	82.9	88.7	91.4	95.9	103.6	107.3	114.4	117.8	123.9	131.2	135.4	140.5	145.1
12762	90.2	97.5	101.2	109.0	114.0	121.6	125.8	130.2	140.1	144.6	146.1	150.3	156.9
12763	93.8	102.2	104.5	109.1	120.2	124.3	131.0	138.0	145.6	152.2	158.8	164.3	170.9
12764	109.3	119.7	122.6	128.9	134.3	139.1	145.7	153.3	159.4	163.1	171.3	172.9	179.5
12765	105.4	111.2	114.6	124.8	130.4	135.2	140.4	153.6	161.0	162.9	168.9	174.7	179.6
12766	89.4	92.2	95.6	103.8	106.2	110.9	118.4	126.6	132.6	138.5	140.9	145.0	151.4
12767	97.2	102.4	105.3	114.2	123.7	131.4	137.0	142.0	149.6	159.0	163.0	169.2	176.6
12768	90.8	94.4	98.6	107.5	113.2	116.4	124.3	132.6	139.6	139.8	145.0	148.4	155.6

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 8): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP II				LOW DOSAGE				100 PPM				
	DAY 27	28	29	30	31	32	33	34	35	36	37	38	39
12753	203.8	213.9	218.8	226.4	228.6	235.5	245.9	256.6	260.7	261.4	267.7	273.3	283.3
12754	213.8	215.9	223.7	229.3	236.5	237.3	245.7	251.7	257.5	266.1	269.9	274.7	287.3
12755	153.1	158.5	162.4	166.4	167.8	175.1	179.9	184.8	193.6	196.5	199.1	206.8	209.9
12756	175.5	176.2	183.8	187.6	192.1	195.8	202.7	205.2	212.9	219.6	226.2	226.6	233.3
12757	177.7	183.1	186.2	188.9	190.3	196.8	202.3	206.3	212.0	211.2	218.7	216.8	227.0
12758	185.6	189.3	196.2	195.7	201.0	206.9	212.0	218.7	224.0	228.8	229.0	229.9	236.5
12759	180.4	185.9	188.5	198.3	198.2	205.9	211.0	216.0	222.0	227.1	232.6	238.2	245.7
12760	181.0	189.1	190.2	195.4	198.9	205.7	214.6	219.0	226.8	228.9	236.8	239.2	250.0
12761	149.7	153.5	160.6	161.8	166.2	169.9	177.0	183.8	190.1	194.2	198.3	204.2	208.7
12762	158.8	161.2	167.8	170.0	176.8	182.2	188.3	194.5	196.6	197.4	208.0	207.8	217.0
12763	181.2	182.5	189.9	188.4	194.7	199.5	205.8	214.4	220.7	222.8	230.5	240.0	239.4
12764	191.6	195.6	199.0	202.6	207.4	212.1	220.8	222.8	229.2	238.6	245.9	252.8	256.2
12765	185.4	190.4	193.8	197.3	205.5	212.5	219.3	225.0	225.6	233.2	235.2	243.1	247.8
12766	156.2	163.1	165.3	169.3	176.2	181.5	186.1	192.4	192.7	197.4	200.5	209.4	212.9
12767	181.8	186.5	190.3	197.3	200.6	208.3	211.8	219.4	223.5	232.8	236.4	244.3	247.1
12768	160.2	163.1	166.8	173.5	180.5	180.9	191.1	191.3	196.0	197.7	202.4	208.2	210.6

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 9): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP II					LOW DOSAGE				100 PPM			
	DAY 40	41	42	43	44	45	46	47	48	49	50	51	52
12753	284.2	291.7	293.7	300.0	300.0	MORIBUND SACRIFICED ON DAY 44 OF STUDY							
12754	291.0	295.7	302.3	300.8	310.9	316.1	316.7	317.4	326.3	323.6	326.5	334.2	337.8
12755	216.5	219.4	219.3	224.8	225.3	226.7	229.0	232.6	231.2	231.0	239.1	237.7	242.2
12756	237.7	242.3	246.1	247.0	251.3	250.3	254.9	258.7	259.6	265.6	267.7	270.8	272.9
12757	225.9	225.1	231.5	228.7	234.8	233.8	235.5	236.0	242.3	242.6	246.2	244.4	247.5
12758	240.0	242.6	247.0	249.7	249.1	253.2	252.4	257.9	257.4	264.8	262.7	267.9	271.7
12759	249.7	253.3	257.7	261.8	263.1	258.7	258.7	261.3	265.0	264.5	264.9	271.7	278.9
12760	249.4	254.0	259.5	263.5	263.0	263.9	267.6	269.2	274.1	276.8	282.0	284.7	291.4
12761	212.5	216.1	222.2	226.4	226.1	229.6	227.6	229.7	235.5	239.8	241.5	244.2	248.1
12762	217.4	222.7	224.4	224.1	230.9	233.7	234.9	239.4	238.7	243.0	245.8	248.0	252.6
12763	250.3	249.7	258.3	259.0	265.4	268.9	275.0	280.7	286.5	289.8	292.4	298.9	303.6
12764	265.5	266.7	270.7	272.4	285.7	283.6	289.7	293.5	296.4	299.4	302.0	309.2	308.4
12765	252.2	253.8	255.7	262.3	265.0	268.7	272.2	273.4	283.8	282.5	286.4	288.2	289.2
12766	216.5	219.8	220.7	228.4	224.1	228.3	233.2	235.3	242.0	241.4	249.0	250.2	249.7
12767	252.0	260.0	259.7	269.2	270.8	272.6	276.8	281.1	286.6	288.0	292.4	296.5	299.1
12768	212.9	217.5	218.3	223.3	224.6	228.1	234.9	237.3	239.1	244.6	246.6	245.3	249.7

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 10): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP II				LOW DOSAGE			100 PPM	
	DAY 53	54	55	56	57	58	59	60	61
12753	MORIBUND SACRIFICED ON DAY 44 OF STUDY								
12754	342.9	347.4	350.9	354.0	356.8	361.4	360.0		
12755	244.9	245.9	249.7	254.0	258.7	259.9	255.2		
12756	272.3	274.8	279.7	279.6	281.0	286.6	284.4		
12757	250.2	252.6	250.9	256.2	261.6	258.7	256.9		
12758	273.1	276.0	284.0	282.5	285.7	290.9	288.9		
12759	278.2	285.4	282.3	288.5	296.1	295.9	292.6	297.7	
12760	296.7	297.9	301.6	302.3	307.9	307.7	308.6	311.0	
12761	252.1	252.0	255.9	259.9	258.5	263.3	266.9	266.5	
12762	253.0	255.6	261.3	263.1	262.4	266.5	264.0	265.5	
12763	304.9	309.4	315.5	317.0	320.7	314.6	313.1	321.5	
12764	312.3	320.5	321.7	324.2	327.0	326.6	329.6	331.6	
12765	294.8	300.0	300.6	301.8	308.8	305.5	310.3	312.5	
12766	251.3	256.2	259.5	260.4	264.2	260.9	265.8	265.0	
12767	304.8	307.9	310.4	312.1	318.3	315.0	321.2	323.8	
12768	251.9	264.1	265.1	265.4	268.3	265.0	266.6	271.4	

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 11): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP III					MIDDLE DOSAGE				1000 PPM			
DAY	1	2	3	4	5	6	7	8	9	10	11	12	13
12769	41.5	43.3	47.9	52.9	56.3	62.6	66.9	71.1	77.4	82.7	89.5	96.0	102.6
12770	42.0	46.4	50.2	56.5	61.1	66.1	70.5	76.3	80.0	88.5	94.5	100.2	105.8
12771	39.4	36.8	40.6	45.9	49.9	55.9	59.9	63.4	68.6	74.4	82.7	89.3	94.9
12772	46.6	46.2	50.3	56.1	63.7	69.1	74.2	77.6	82.9	90.7	95.9	104.6	111.3
12773	38.8	42.5	47.0	53.8	60.8	66.5	66.4	71.6	79.6	85.3	93.9	101.4	111.0
12774	33.3	35.3	39.8	46.0	50.2	55.7	60.0	64.4	67.5	69.1	75.4	82.8	88.9
12775	32.4	33.8	40.3	43.9	49.2	54.1	58.9	62.4	65.9	69.3	75.2	79.8	85.5
12776	38.4	41.8	46.6	52.6	56.6	62.5	67.9	73.2	79.2	86.4	91.8	97.8	107.2
12777	34.9	38.8	42.2	48.1	54.8	60.5	66.5	72.6	78.2	85.2	90.5	98.8	104.3
12778	28.3	29.3	30.9	32.5	37.3	42.8	46.9	50.4	53.6	59.0	63.6	68.3	73.7
12779	42.2	43.6	47.1	53.2	58.1	63.9	68.8	73.2	76.9	83.7	91.1	97.3	103.1
12780	39.9	40.6	44.7	51.1	55.2	60.7	66.5	70.2	74.8	81.6	87.8	94.4	99.5
12781	33.1	32.2	33.1	35.2	41.2	43.3	46.4	50.4	54.4	58.3	63.9	69.0	73.8
12782	37.3	32.9	39.8	45.4	50.7	55.3	58.2	63.4	68.8	74.7	80.5	88.3	94.6
12783	39.7	39.5	44.0	47.8	54.9	59.1	63.6	69.0	77.4	80.6	86.9	93.5	100.6
12784	40.0	37.4	40.6	44.7	48.5	53.4	57.9	62.6	68.3	73.6	79.0	85.3	90.4

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 12): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP III					MIDDLE DOSAGE				1000 PPM			
	DAY 14	15	16	17	18	19	20	21	22	23	24	25	26
12769	110.4	116.9	123.5	129.5	138.6	147.6	153.6	157.3	165.0	172.0	176.3	181.1	188.3
12770	111.9	116.6	125.1	131.6	140.8	146.0	152.6	157.6	164.5	170.5	175.6	180.9	185.2
12771	99.5	109.6	110.4	119.3	127.7	131.0	138.4	145.3	154.4	159.3	164.3	171.9	178.4
12772	118.0	125.5	128.6	138.2	146.1	149.8	155.9	164.4	171.3	177.0	182.0	188.6	196.5
12773	117.6	122.4	125.3	135.2	145.6	152.0	157.0	162.5	173.1	180.5	186.2	190.3	196.4
12774	96.7	102.4	104.9	109.6	118.1	123.4	123.1	132.9	138.5	144.9	151.4	155.1	160.9
12775	92.0	98.1	100.8	107.4	115.6	119.2	129.7	132.7	138.1	143.8	148.8	153.6	157.6
12776	115.3	120.3	124.8	133.3	140.3	145.3	154.1	160.1	168.7	175.0	181.9	186.6	191.5
12777	110.2	117.5	121.3	129.8	138.6	145.9	152.0	157.5	166.9	172.0	177.7	183.6	193.2
12778	79.9	84.6	87.6	93.2	99.8	105.6	112.6	117.3	126.8	131.1	136.9	143.2	147.4
12779	109.8	118.0	120.4	124.6	134.6	140.5	144.1	150.6	157.4	163.3	170.3	173.9	178.3
12780	105.3	112.1	114.5	119.0	130.0	134.2	143.4	147.5	155.0	161.0	168.0	171.4	177.4
12781	80.5	84.7	89.4	94.3	101.5	105.8	111.1	122.5	127.7	129.9	135.9	145.7	147.3
12782	102.0	106.9	112.6	120.9	126.5	136.3	142.3	150.1	156.8	161.1	167.2	170.8	177.3
12783	109.5	113.5	122.8	128.9	134.5	141.9	147.9	155.5	163.7	168.3	175.9	179.9	185.3
12784	97.0	98.6	105.6	113.2	120.5	126.3	132.0	141.1	144.8	150.5	157.9	162.3	166.1

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 13): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP III					MIDDLE DOSAGE				1000 PPM			
	DAY 27	28	29	30	31	32	33	34	35	36	37	38	39
12769	194.3	199.8	203.9	209.7	217.1	220.9	226.7	232.4	239.6	242.9	250.9	257.2	261.7
12770	191.3	197.4	201.3	206.2	213.6	220.5	222.1	229.2	238.4	239.2	248.3	249.4	256.1
12771	185.7	190.0	196.2	200.2	207.5	214.8	219.9	229.1	234.2	236.3	244.8	246.1	257.4
12772	202.1	210.7	217.6	223.4	227.2	234.1	243.7	248.7	257.0	260.6	266.6	270.8	280.9
12773	202.0	208.0	211.9	215.4	225.5	233.8	239.2	248.2	255.5	253.3	266.6	265.6	275.0
12774	166.9	170.0	177.7	182.2	185.2	189.9	192.6	201.3	207.0	209.0	216.8	220.8	228.5
12775	165.4	172.7	178.3	182.5	186.7	191.9	201.9	207.7	211.9	215.4	225.8	229.8	239.5
12776	199.6	205.4	210.9	213.7	220.4	225.4	235.2	236.5	246.0	249.9	256.5	262.6	271.6
12777	197.3	201.1	208.7	210.4	219.5	224.2	233.8	241.4	248.1	248.7	258.3	259.0	266.4
12778	152.4	155.4	160.7	164.6	169.8	176.2	179.1	183.7	193.5	192.3	198.7	198.8	208.5
12779	188.3	189.6	197.9	201.5	206.0	211.6	217.6	221.6	231.2	230.9	238.1	242.7	248.0
12780	186.5	193.9	195.3	197.0	203.6	213.0	220.2	224.6	232.4	233.0	245.0	247.6	254.1
12781	152.4	160.7	161.9	167.5	174.5	176.3	190.2	192.5	196.2	203.1	206.4	213.7	219.8
12782	180.1	186.8	190.2	196.5	195.2	200.0	210.8	217.3	216.9	219.3	227.5	231.1	234.2
12783	192.5	197.9	204.6	209.6	216.3	224.0	234.6	242.9	242.2	248.0	250.6	260.4	262.8
12784	172.6	178.9	179.5	185.0	188.9	195.2	198.5	205.7	205.2	211.3	214.2	220.8	224.7

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 14): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP III					MIDDLE DOSAGE				1000 PPM			
	DAY 40	41	42	43	44	45	46	47	48	49	50	51	52
12769	264.3	268.3	272.3	273.5	280.4	284.4	285.0	288.0	291.2	292.9	298.4	304.7	302.8
12770	257.9	262.8	270.0	272.8	274.3	281.5	279.0	284.9	285.6	287.6	290.8	293.0	297.2
12771	260.6	268.7	270.6	275.7	281.0	282.8	285.5	298.6	297.4	300.2	306.1	307.8	311.0
12772	286.6	288.7	292.7	299.0	301.9	305.9	307.8	307.8	314.3	315.4	323.9	325.4	332.8
12773	275.6	283.7	287.3	291.7	293.5	293.4	298.3	306.9	310.4	315.3	320.2	317.4	319.8
12774	227.6	230.7	238.9	238.5	241.1	243.2	247.1	248.2	254.5	254.9	259.9	259.0	261.8
12775	239.9	246.3	252.6	251.7	257.6	261.5	263.1	266.8	274.4	272.7	274.7	279.1	285.8
12776	275.4	277.6	284.4	283.8	289.3	290.6	291.8	297.0	300.8	306.6	307.9	313.4	315.1
12777	269.2	275.1	281.2	281.7	284.8	291.1	301.8	299.7	306.5	312.8	314.3	314.8	322.7
12778	209.3	213.9	216.4	217.6	223.2	221.4	225.4	227.9	230.1	231.7	234.0	233.6	237.4
12779	249.6	258.0	261.7	261.7	266.1	263.8	266.2	270.7	276.9	277.9	280.4	279.0	283.1
12780	260.2	263.2	268.2	270.5	272.9	274.2	281.1	279.2	293.1	287.8	293.5	297.4	304.6
12781	223.6	228.0	230.9	237.3	237.1	241.3	246.6	250.9	254.6	256.0	257.8	261.2	262.9
12782	236.9	241.2	247.7	249.4	246.0	248.5	252.3	256.5	262.5	261.0	261.0	266.0	266.7
12783	268.6	273.6	277.2	286.1	283.8	286.7	294.9	300.0	305.4	307.3	309.0	316.1	320.5
12784	228.4	230.5	234.6	234.0	236.9	238.8	244.1	244.8	247.3	248.5	251.6	256.0	256.6

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 15): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP III				MIDDLE DOSAGE			1000 PPM	
	DAY 53	54	55	56	57	58	59	60	61
12769	309.4	313.4	314.7	315.5	324.8	324.4	327.7		
12770	300.0	300.9	306.0	308.1	309.5	313.0	312.3		
12771	315.4	319.9	327.5	329.8	330.5	334.4	333.0		
12772	334.5	340.7	343.5	345.4	351.1	355.0	352.8		
12773	330.7	330.4	338.6	344.5	339.0	347.7	350.6		
12774	264.1	269.6	269.0	275.2	277.8	277.6	276.1		
12775	284.8	292.6	295.4	299.5	299.6	303.2	303.4	306.4	
12776	316.6	327.9	324.8	331.9	334.0	335.4	331.1	335.6	
12777	327.8	329.0	331.3	337.6	343.5	344.4	346.3	349.2	
12778	240.0	241.2	244.2	247.4	252.1	249.2	251.1	253.5	
12779	288.8	292.9	291.8	295.7	301.4	299.7	302.0	305.4	
12780	305.0	310.6	309.0	315.8	321.2	319.6	324.1	322.0	326.1
12781	264.5	267.1	269.6	268.3	271.5	271.4	276.5	274.4	
12782	269.6	273.1	272.0	276.4	280.0	276.7	280.4	279.3	
12783	323.6	330.4	335.2	334.4	342.1	342.5	350.3	351.3	
12784	256.2	257.3	259.2	262.1	264.0	265.4	265.4	266.9	

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 16): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP IV					HIGH DOSAGE				10000 PPM			
	DAY 14	15	16	17	18	19	20	21	22	23	24	25	26
12785	100.8	108.4	110.2	115.2	121.6	127.3	129.7	135.3	141.9	145.7	152.4	155.8	159.5
12786	108.0	113.0	116.5	124.0	129.0	134.0	139.0	146.0	151.5	155.5	159.6	164.7	170.1
12787	117.8	123.8	127.6	135.5	146.1	148.6	154.6	159.4	169.5	172.5	180.7	185.9	192.1
12788	85.0	90.0	92.5	98.6	107.4	110.8	119.7	124.0	132.1	135.0	146.6	146.6	149.5
12789	102.8	109.4	111.8	117.0	123.6	132.7	137.7	143.3	152.1	154.6	162.2	168.0	171.4
12790	110.9	119.4	123.3	128.5	138.4	147.2	154.4	161.6	172.3	173.2	183.1	191.1	195.9
12791	93.1	97.3	102.2	103.1	111.5	117.1	123.0	129.0	132.1	137.3	147.6	150.2	154.5
12792	103.7	110.3	116.2	121.6	130.8	135.8	139.9	149.4	155.9	164.8	169.4	176.7	184.1
12793	83.3	88.8	89.9	96.3	104.5	109.6	117.9	123.0	131.4	137.4	143.8	148.0	152.9
12794	101.1	109.6	112.5	119.8	128.1	131.6	139.2	145.5	153.8	157.5	165.8	169.0	175.1
12795	97.8	102.4	106.2	113.5	121.5	125.8	134.5	140.3	149.6	152.0	159.8	164.1	169.6
12796	117.3	124.7	130.0	134.8	144.0	150.4	156.6	162.4	168.6	174.3	181.7	186.4	193.3
12797	75.2	77.4	84.2	89.4	93.7	99.2	106.9	114.4	119.3	123.2	128.1	134.9	139.7
12798	81.3	85.6	89.8	98.1	103.3	110.1	117.0	124.0	127.7	129.8	137.2	142.1	147.1
12799	102.3	106.3	112.6	122.5	124.1	131.3	137.9	146.9	154.8	159.4	165.7	173.9	177.7
12800	109.2	111.5	117.1	126.3	133.4	139.6	146.4	154.7	162.1	165.1	173.0	176.1	184.6

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 17): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP IV					HIGH DOSAGE				10000 PPM			
	DAY 27	28	29	30	31	32	33	34	35	36	37	38	39
12785	168.1	169.5	177.7	177.8	180.7	189.0	195.9	200.6	206.0	208.3	212.4	216.5	225.4
12786	173.8	177.7	185.6	186.2	187.4	194.1	197.4	204.8	208.9	210.5	214.9	218.6	226.0
12787	200.1	203.4	208.8	212.6	217.6	226.1	227.0	233.7	242.3	240.2	248.1	251.5	260.7
12788	158.4	162.5	168.1	166.0	178.7	178.7	187.9	187.7	195.4	199.7	203.6	207.1	217.2
12789	179.5	183.5	190.5	191.5	202.3	200.7	209.3	213.8	224.9	225.6	230.7	234.3	241.6
12790	201.1	211.0	214.6	220.9	226.3	232.1	239.5	242.5	253.4	254.8	264.0	264.4	273.6
12791	162.3	167.4	171.3	175.1	178.2	183.3	189.8	193.2	203.4	206.9	208.9	213.5	221.8
12792	191.8	195.6	201.9	209.3	212.8	220.9	226.7	235.1	246.9	243.3	251.1	253.6	261.8
12793	158.8	166.1	171.1	176.0	180.1	184.2	189.6	197.2	207.2	208.0	214.2	217.4	224.6
12794	179.4	185.3	189.1	194.1	200.0	205.4	211.1	218.9	221.7	224.5	234.6	235.7	243.6
12795	174.0	171.4	178.3	182.4	187.9	195.0	198.3	205.7	211.6	219.5	226.7	232.5	238.4
12796	198.5	203.6	209.5	217.7	220.7	226.6	232.0	235.0	243.7	247.4	254.1	257.1	263.5
12797	146.2	152.4	153.5	161.0	168.2	171.3	174.0	182.8	184.7	193.2	195.6	204.5	207.2
12798	154.1	161.2	155.8	166.3	168.2	174.3	180.8	187.5	185.2	192.0	196.5	204.0	204.5
12799	185.5	189.3	197.5	199.5	207.5	212.1	221.5	226.7	230.9	236.4	240.9	249.8	250.8
12800	187.4	196.7	197.0	202.9	206.9	212.4	217.0	222.5	228.4	233.4	237.1	241.9	247.6

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 18): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP IV					HIGH DOSAGE				10000 PPM			
	DAY 40	41	42	43	44	45	46	47	48	49	50	51	52
12785	227.5	232.8	237.7	239.1	241.6	243.0	245.8	247.7	249.3	256.7	261.3	262.3	264.5
12786	227.3	229.8	239.3	238.5	242.2	243.9	248.4	253.1	253.6	255.0	260.8	262.4	266.2
12787	257.8	259.3	267.6	267.4	271.0	270.6	274.1	279.4	280.5	289.6	290.3	292.3	297.4
12788	221.3	222.9	227.4	230.1	232.8	234.1	236.7	239.8	243.2	243.9	248.0	251.1	255.0
12789	244.3	252.9	255.0	257.2	259.1	257.5	263.8	268.7	273.3	275.1	279.3	281.2	283.2
12790	276.7	280.0	285.7	291.9	294.3	297.7	304.9	308.3	310.4	316.7	320.8	325.1	325.8
12791	224.7	228.8	235.0	235.6	237.8	243.8	243.5	244.5	251.1	251.3	256.3	256.8	264.7
12792	266.5	272.2	276.9	279.1	287.2	287.1	291.3	300.4	304.1	307.3	314.7	313.9	319.4
12793	229.8	235.3	238.3	243.0	249.4	250.4	254.7	260.2	264.5	269.7	270.5	273.5	281.4
12794	243.7	246.2	253.7	252.3	256.1	261.5	263.0	266.5	265.7	275.0	276.5	277.2	281.3
12795	241.4	243.5	252.9	255.1	262.3	261.3	268.4	270.3	276.5	280.4	285.0	285.3	295.2
12796	267.1	268.9	279.1	283.7	289.4	288.5	296.1	296.9	304.1	306.1	309.9	311.1	318.4
12797	216.1	218.7	219.6	228.0	228.6	229.6	235.2	236.5	239.7	245.1	245.7	255.8	255.1
12798	210.9	217.5	215.5	219.7	220.6	224.7	225.7	229.9	225.5	231.1	230.4	236.4	237.4
12799	258.0	265.9	266.5	275.7	272.1	273.5	278.9	282.4	288.8	293.4	295.0	297.5	303.0
12800	249.8	256.2	258.6	261.9	264.6	266.0	269.9	277.6	278.3	283.1	285.0	285.0	288.2

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 16 (PAGE 19): BODY WEIGHTS - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP IV				HIGH DOSAGE			10000 PPM	
	DAY 53	54	55	56	57	58	59	60	61
12785	270.3	272.9	278.2	280.1	283.4	286.6	288.5		
12786	270.3	270.9	276.9	276.8	278.4	283.7	278.0		
12787	297.6	298.6	305.1	309.4	309.0	314.1	311.7		
12788	258.0	260.2	262.0	261.4	267.8	268.5	268.3		
12789	286.4	286.7	298.1	293.6	298.9	300.1	300.0		
12790	331.2	335.4	338.6	343.6	349.8	348.7	351.9	352.5	
12791	265.4	265.3	266.9	270.1	273.5	277.4	276.4	280.5	
12792	322.3	324.4	325.1	330.9	332.9	338.5	340.1	339.9	
12793	282.5	287.7	289.8	297.3	297.8	299.9	299.2	308.3	
12794	284.5	285.7	290.2	292.6	295.2	300.1	301.9	299.8	
12795	292.3	298.1	302.0	308.6	309.6	314.6	310.2	314.7	312.7
12796	320.4	325.0	322.9	327.7	334.0	335.8	336.8	336.7	342.3
12797	257.8	262.8	260.1	266.3	268.8	265.7	265.3	270.5	
12798	237.7	241.4	241.5	242.8	243.3	245.9	244.8	235.4	
12799	305.4	313.9	316.8	316.0	325.0	324.5	328.9	334.1	
12800	288.3	290.2	295.8	299.9	296.3	299.0	296.6	297.5	

DAY = DAY OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 17 (PAGE 1): FEED CONSUMPTION VALUES - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP I				CARRIER CONTROL				0 PPM			
DAYS	1 - 4	4 - 8	8 - 11	11 - 15	15 - 18	18 - 22	22 - 25	25 - 29	29 - 32	32 - 36	36 - 39	39 - 43	43 - 46
12737	22.	50.	44.	67.	52.	82.	65.	91.	61.	92.	65.	91.	61.
12738	19.	39.	31.	60.	51.	79.	63.	88.	55.	83.	66.	89.	58.
12739	16.	46.	41.	62.	49.	78.	63.	85.	61.	87.	68.	93.	71.
12740	19.	55.	44.	69.	53.	86.	69.	99.	66.	101.	68.	107.	70.
12741	19.	55.	45.	75.	56.	88.	67.	100.	53.	a			
12742	19.	47.	40.	69.	387b	85.	67.	94.	61.	86.	61.	91.	58.
12743	19.	44.	42.	65.	48.	79.	65.	95.	63.	95.	68.	102.	63.
12744	23.	45.	40.	67.	53.	84.	65.	101.	67.	99.	74.	102.	72.
12745	22.	57.	43.	68.	53.	89.	68.	101.	69.	98.	73.	101.	68.
12746	24.	51.	41.	65.	53.	81.	65.	92.	55.	88.	62.	93.	63.
12747	19.	41.	37.	67.	46.	76.	59.	89.	57.	315b	63.	95.	62.
12748	20.	48.	44.	73.	56.	83.	67.	100.	65.	101.	71.	556b	67.
12749	15.	42.	39.	62.	44.	75.	58.	88.	61.	111.	66.	88.	57.
12750	22.	50.	46.	75.	56.	90.	72.	100.	74.	99.	77.	103.	75.
12751	16.	47.	45.	68.	51.	82.	65.	92.	63.	89.	68.	93.	62.
12752	17.	46.	42.	70.	49.	82.	66.	92.	65.	93.	79.	98.	70.

DAYS = DAYS OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Rat 12741 was moribund sacrificed on day 32 of study.
b. Value appeared incorrectly recorded and was excluded from group averages and statistical analyses.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 17 (PAGE 2): FEED CONSUMPTION VALUES - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP I	CARRIER CONTROL	0 PPM
	DAYS 46 - 50	50 - 53	53 - 57
12737	84.	68.	88.
12738	84.	72.	95.
12739	96.	74.	92.
12740	85.	78.	102.
12741	MORIBUND SACRIFICED ON DAY 32 OF STUDY		
12742	84.	66.	92.
12743	84.	74.	87.
12744	98.	74.	104.
12745	93.	77.	95.
12746	86.	67.	86.
12747	85.	67.	88.
12748	88.	75.	94.
12749	78.	51.	67.
12750	96.	75.	94.
12751	95.	69.	87.
12752	102.	71.	94.

DAYS = DAYS OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 17 (PAGE 3): FEED CONSUMPTION VALUES - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP II			LOW DOSAGE			100 PPM					
DAYS	1 - 4	4 - 8	8 - 11	11 - 15	15 - 18	18 - 22	22 - 25	25 - 29	29 - 32	32 - 36	36 - 39	39 - 43	43 - 46
12753	19.	50.	46.	73.	61.	98.	76.	114.	77.	112.	82.	118.	a
12754	21.	50.	44.	75.	59.	92.	71.	105.	71.	105.	76.	107.	76.
12755	10.	38.	32.	53.	43.	72.	58.	82.	56.	84.	65.	94.	58.
12756	16.	45.	38.	62.	47.	83.	69.	96.	64.	96.	67.	101.	68.
12757	16.	41.	36.	62.	50.	79.	63.	88.	57.	87.	55.	81.	55.
12758	24.	45.	44.	77.	49.	80.	65.	96.	63.	90.	58.	94.	59.
12759	15.	38.	43.	66.	50.	78.	65.	100.	68.	104.	75.	106.	59.
12760	20.	41.	43.	74.	52.	85.	68.	101.	67.	101.	75.	106.	61.
12761	13.	34.	32.	54.	40.	65.	53.	78.	51.	81.	55.	88.	48.
12762	17.	41.	32.	57.	46.	71.	52.	80.	52.	80.	58.	79.	54.
12763	12.	35.	37.	63.	46.	77.	63.	95.	60.	94.	65.	104.	66.
12764	20.	51.	42.	69.	51.	82.	62.	96.	62.	101.	68.	107.	69.
12765	20.	46.	43.	69.	50.	85.	66.	96.	68.	96.	72.	106.	72.
12766	13.	42.	43.	61.	40.	71.	54.	83.	58.	79.	64.	86.	58.
12767	12.	39.	38.	63.	50.	81.	64.	88.	62.	91.	68.	92.	68.
12768	15.	40.	41.	62.	47.	82.	61.	88.	61.	82.	65.	85.	60.

DAYS = DAYS OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Rat 12753 was moribund sacrificed on day 44 of study.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 17 (PAGE 4): FEED CONSUMPTION VALUES - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP II	LOW DOSAGE	100 PPM
	DAYS 46 - 50	50 - 53	53 - 57
12753	MORIBUND SACRIFICED ON DAY 44 OF STUDY		
12754	92.	82.	100.
12755	78.	60.	84.
12756	88.	71.	88.
12757	76.	60.	78.
12758	79.	68.	88.
12759	74.	71.	99.
12760	84.	73.	94.
12761	72.	63.	a
12762	74.	59.	76.
12763	93.	73.	99.
12764	95.	76.	101.
12765	102.	77.	95.
12766	82.	59.	76.
12767	97.	69.	90.
12768	91.	62.	87.

DAYS = DAYS OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Spilled feed precluded the calculation of this value.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 17 (PAGE 5): FEED CONSUMPTION VALUES - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP III				MIDDLE DOSAGE			1000 PPM				
DAYS	1 - 4	4 - 8	8 - 11	11 - 15	15 - 18	18 - 22	22 - 25	25 - 29	29 - 32	32 - 36	36 - 39	39 - 43	43 - 46
12769	18.	42.	42.	70.	54.	81.	63.	97.	63.	95.	63.	96.	66.
12770	21.	48.	42.	64.	54.	79.	61.	98.	67.	94.	66.	97.	65.
12771	14.	47.	39.	67.	48.	82.	63.	94.	63.	95.	68.	101.	67.
12772	20.	53.	43.	70.	55.	82.	64.	98.	67.	103.	68.	104.	66.
12773	23.	46.	44.	80.	58.	88.	68.	102.	69.	102.	68.	111.	69.
12774	20.	47.	38.	80.	50.	79.	64.	95.	60.	96.	63.	95.	63.
12775	18.	42.	37.	60.	45.	74.	60.	90.	60.	98.	71.	99.	70.
12776	22.	47.	43.	74.	52.	87.	71.	100.	66.	105.	72.	102.	a
12777	22.	51.	44.	76.	59.	94.	79.	104.	72.	112.	77.	115.	79.
12778	12.	37.	35.	54.	46.	65.	53.	82.	58.	87.	72.	93.	57.
12779	19.	51.	47.	81.	51.	81.	63.	97.	61.	97.	63.	98.	60.
12780	19.	44.	37.	60.	54.	81.	63.	96.	61.	95.	67.	100.	64.
12781	12.	39.	37.	61.	43.	80.	67.	91.	69.	103.	80.	113.	87.
12782	16.	42.	41.	68.	49.	84.	63.	87.	66.	98.	70.	91.	60.
12783	18.	48.	50.	77.	59.	93.	77.	110.	73.	100.	78.	104.	73.
12784	13.	41.	42.	61.	50.	76.	62.	82.	59.	81.	59.	90.	77.

DAYS = DAYS OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Spilled feed precluded the calculation of this value.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 17 (PAGE 6): FEED CONSUMPTION VALUES - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP III	MIDDLE DOSAGE	1000 PPM
	DAYS 46 - 50	50 - 53	53 - 57
12769	88.	69.	94.
12770	88.	70.	90.
12771	95.	76.	96.
12772	92.	78.	99.
12773	100.	79.	101.
12774	87.	a	92.
12775	91.	73.	97.
12776	91.	72.	96.
12777	110.	a	116.
12778	81.	61.	83.
12779	83.	63.	83.
12780	91.	72.	94.
12781	a	93.	123.
12782	89.	70.	82.
12783	106.	78.	103.
12784	79.	73.	81.

DAYS = DAYS OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Spilled feed precluded the calculation of this value.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 17 (PAGE 7): FEED CONSUMPTION VALUES - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP IV					HIGH DOSAGE				10000 PPM			
DAYS	1 - 4	4 - 8	8 - 11	11 - 15	15 - 18	18 - 22	22 - 25	25 - 29	29 - 32	32 - 36	36 - 39	39 - 43	43 - 46
12785	17.	46.	42.	66.	50.	81.	62.	95.	59.	90.	66.	96.	63.
12786	17.	44.	40.	64.	50.	76.	65.	92.	61.	86.	61.	90.	61.
12787	22.	53.	39.	68.	52.	76.	67.	94.	63.	93.	70.	99.	60.
12788	11.	31.	34.	54.	43.	69.	54.	82.	51.	81.	54.	80.	53.
12789	17.	43.	46.	70.	48.	76.	60.	90.	59.	96.	63.	95.	60.
12790	25.	52.	48.	74.	56.	96.	81.	127.	84.	125.	86.	136.	a
12791	16.	43.	36.	64.	42.	65.	54.	86.	55.	92.	62.	96.	60.
12792	20.	45.	39.	64.	54.	92.	76.	109.	74.	106.	75.	111.	78.
12793	13.	33.	38.	56.	40.	66.	54.	84.	56.	87.	63.	96.	63.
12794	19.	46.	45.	66.	52.	77.	61.	89.	59.	89.	a	85.	60.
12795	15.	41.	34.	56.	44.	72.	58.	77.	54.	85.	63.	93.	63.
12796	17.	48.	44.	77.	57.	88.	69.	102.	67.	103.	70.	110.	73.
12797	17.	40.	25.	59.	40.	71.	58.	82.	59.	82.	64.	86.	63.
12798	10.	29.	36.	65.	48.	79.	69.	89.	66.	83.	66.	90.	65.
12799	18.	48.	46.	73.	52.	87.	69.	100.	70.	107.	76.	130.	71.
12800	15.	48.	37.	72.	50.	79.	64.	91.	61.	83.	67.	87.	62.

DAYS = DAYS OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Spilled feed precluded the calculation of this value.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 17 (PAGE 8): FEED CONSUMPTION VALUES - INDIVIDUAL DATA - MALE RATS

RAT #	DOSAGE GROUP IV	HIGH DOSAGE	10000 PPM
	DAYS 46 - 50	50 - 53	53 - 57
12785	88.	72.	95.
12786	85.	67.	83.
12787	94.	76.	102.
12788	76.	61.	79.
12789	85.	68.	85.
12790	a	a	a
12791	82.	65.	88.
12792	102.	85.	103.
12793	90.	68.	92.
12794	83.	64.	86.
12795	86.	66.	90.
12796	97.	80.	100.
12797	90.	69.	85.
12798	85.	65.	79.
12799	117.	84.	108.
12800	90.	64.	81.

DAYS = DAYS OF STUDY
ALL WEIGHTS WERE RECORDED IN GRAMS (G).
a. Spilled feed precluded the calculation of this value.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 18 (PAGE 1): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP DOSAGE (PPM)	RAT NUMBER	DAY OF NECROPSY	OBSERVATIONS a
I			
0	12737	DS 59	ALL TISSUES APPEARED NORMAL.
	12738	DS 59	ALL TISSUES APPEARED NORMAL.
	12739	DS 59	ALL TISSUES APPEARED NORMAL.
	12740	DS 59	ALL TISSUES APPEARED NORMAL.
	12741	DS 32	MORIBUND SACRIFICED ON DAY 32 OF STUDY. EYES: RIGHT, ANTERIOR CHAMBER OF EYE COLLAPSED. ALL OTHER TISSUES APPEARED NORMAL.
	12742	DS 59	ALL TISSUES APPEARED NORMAL.
	12743	DS 60	ALL TISSUES APPEARED NORMAL.
	12744	DS 60	ALL TISSUES APPEARED NORMAL.
	12745	DS 60	ALL TISSUES APPEARED NORMAL.
	12746	DS 60	ALL TISSUES APPEARED NORMAL.
	12747	DS 60	ALL TISSUES APPEARED NORMAL.
	12748	DS 61	ALL TISSUES APPEARED NORMAL.
	12749	DS 60	ALL TISSUES APPEARED NORMAL.
	12750	DS 60	ALL TISSUES APPEARED NORMAL.
	12751	DS 60	ALL TISSUES APPEARED NORMAL.
	12752	DS 60	ALL TISSUES APPEARED NORMAL.

DS = DAY OF STUDY

a. Refer to the individual clinical observations table (Table 14) for external observations confirmed at necropsy.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 18 (PAGE 2): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP DOSAGE (PPM)	RAT NUMBER	DAY OF NECROPSY	OBSERVATIONS a
II 100	12753	DS 44	MORIBUND SACRIFICED ON DAY 44 OF STUDY. ALL TISSUES APPEARED NORMAL.
	12754	DS 59	ALL TISSUES APPEARED NORMAL.
	12755	DS 59	ALL TISSUES APPEARED NORMAL.
	12756	DS 59	ALL TISSUES APPEARED NORMAL.
	12757	DS 59	ALL TISSUES APPEARED NORMAL.
	12758	DS 59	ALL TISSUES APPEARED NORMAL.
	12759	DS 60	ALL TISSUES APPEARED NORMAL.
	12760	DS 60	ALL TISSUES APPEARED NORMAL.
	12761	DS 60	ALL TISSUES APPEARED NORMAL.
	12762	DS 60	ALL TISSUES APPEARED NORMAL.
	12763	DS 60	ALL TISSUES APPEARED NORMAL.
	12764	DS 60	ALL TISSUES APPEARED NORMAL.
	12765	DS 60	ALL TISSUES APPEARED NORMAL.
	12766	DS 60	ALL TISSUES APPEARED NORMAL.
	12767	DS 60	ALL TISSUES APPEARED NORMAL.
	12768	DS 60	ALL TISSUES APPEARED NORMAL.

DS = DAY OF STUDY
a. Refer to the individual clinical observations table (Table 14) for external observations confirmed at necropsy.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 18 (PAGE 3): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP DOSAGE (PPM)	RAT NUMBER	DAY OF NECROPSY	OBSERVATIONS a
III 1000	12769	DS 59	ALL TISSUES APPEARED NORMAL.
	12770	DS 59	ALL TISSUES APPEARED NORMAL.
	12771	DS 59	ALL TISSUES APPEARED NORMAL.
	12772	DS 59	ALL TISSUES APPEARED NORMAL.
	12773	DS 59	ALL TISSUES APPEARED NORMAL.
	12774	DS 59	ALL TISSUES APPEARED NORMAL.
	12775	DS 60	ALL TISSUES APPEARED NORMAL.
	12776	DS 60	ALL TISSUES APPEARED NORMAL.
	12777	DS 60	ALL TISSUES APPEARED NORMAL.
	12778	DS 60	ALL TISSUES APPEARED NORMAL.
	12779	DS 60	ALL TISSUES APPEARED NORMAL.
	12780	DS 61	ALL TISSUES APPEARED NORMAL.
	12781	DS 60	ALL TISSUES APPEARED NORMAL.
	12782	DS 60	ALL TISSUES APPEARED NORMAL.
	12783	DS 60	ALL TISSUES APPEARED NORMAL.
	12784	DS 60	ALL TISSUES APPEARED NORMAL.

DS = DAY OF STUDY

a. Refer to the individual clinical observations table (Table 14) for external observations confirmed at necropsy.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 18 (PAGE 4): NECROPSY OBSERVATIONS - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP DOSAGE (PPM)	RAT NUMBER	DAY OF NECROPSY	OBSERVATIONS a
IV 10000	12785	DS 59	ALL TISSUES APPEARED NORMAL.
	12786	DS 59	ALL TISSUES APPEARED NORMAL.
	12787	DS 59	ALL TISSUES APPEARED NORMAL.
	12788	DS 59	ALL TISSUES APPEARED NORMAL.
	12789	DS 59	ALL TISSUES APPEARED NORMAL.
	12790	DS 60	ALL TISSUES APPEARED NORMAL.
	12791	DS 60	ALL TISSUES APPEARED NORMAL.
	12792	DS 60	ALL TISSUES APPEARED NORMAL.
	12793	DS 60	ALL TISSUES APPEARED NORMAL.
	12794	DS 60	ALL TISSUES APPEARED NORMAL.
	12795	DS 61	ALL TISSUES APPEARED NORMAL.
	12796	DS 61	ALL TISSUES APPEARED NORMAL.
	12797	DS 60	ALL TISSUES APPEARED NORMAL.
	12798	DS 60	ALL TISSUES APPEARED NORMAL.
	12799	DS 60	ALL TISSUES APPEARED NORMAL.
	12800	DS 60	ALL TISSUES APPEARED NORMAL.

DS = DAY OF STUDY
a. Refer to the individual clinical observations table (Table 14) for external observations confirmed at necropsy.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 19 (PAGE 1): TERMINAL BODY WEIGHTS, ORGAN WEIGHTS AND RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP I — CARRIER CONTROL — 0 PPM

RAT NUMBER	TERMINAL BODY WEIGHT	EPIDIDYMIS LEFT ABS. WT.	EPIDIDYMIS LEFT REL. % TBW	CAUDA EPIDIDYMIS LEFT ABS. WT.	CAUDA EPIDIDYMIS LEFT REL. % TBW	TESTIS LEFT ABS. WT.	TESTIS LEFT REL. % TBW	L. TESTIS MINUS TUNICA ALBUGINEA ABS. WT.	L. TESTIS MINUS TUNICA ALBUGINEA REL. % TBW	SEMINAL VESICLES WITH FLUID ABS. WT.	SEMINAL VESICLES WITH FLUID REL. % TBW	SEMINAL VESICLES WITHOUT FLUID ABS. WT.	SEMINAL VESICLES WITHOUT FLUID REL. % TBW
12737	313.	0.4746	0.15	0.1644	0.05	1.8371	0.59	1.6588	0.53	0.5712	0.18	0.3224	0.10
12738	304.	0.5262	0.17	0.1934	0.06	1.8671	0.61	1.6966	0.56	0.7141	0.23	0.4260	0.14
12739	269.	0.5278	0.20	0.2022	0.08	1.4438	0.54	1.3008	0.48	0.6131	0.23	0.3419	0.13
12740	314.	0.5485	0.17	0.2220	0.07	1.6848	0.54	1.5540	0.49	1.0730	0.34	0.6162	0.20
12741a	----	b	----	------	----	b	----	------	----	------	----	------	----
12742	270.	0.5773	0.21	0.2332	0.09	1.7636	0.65	1.5980	0.59	0.8718	0.32	0.5409	0.20
12743	264.	0.5378	0.20	0.1639	0.06	1.5465	0.58	1.4023	0.53	0.7617	0.29	0.4121	0.16
12744	343.	0.6933	0.20	0.2508	0.07	1.5444	0.45	1.4017	0.41	0.8222	0.24	0.3857	0.11
12745	314.	0.6111	0.19	0.2453	0.08	1.5156	0.48	1.3388	0.42	0.9333	0.30	0.5514	0.18
12746	268.	0.6154	0.23	0.1723	0.06	1.4717	0.55	1.3527	0.50	0.9307	0.35	0.5687	0.21
12747	316.	0.6179	0.20	0.2531	0.08	1.7947	0.57	1.6099	0.51	0.6841	0.22	0.3563	0.11
12748	340.	0.5537	0.16	0.2077	0.06	1.7178	0.50	1.5770	0.46	0.7661	0.22	0.5313	0.16
12749	237.	0.5251	0.22	0.1848	0.08	1.3957	0.59	1.3013	0.55	0.8451	0.36	0.4999	0.21
12750	340.	0.5363	0.16	0.2117	0.06	1.7601	0.52	1.9055	0.56	0.9199	0.27	0.5647	0.17
12751	310.	0.5284	0.17	0.1881	0.06	1.5112	0.49	1.3647	0.44	0.5676	0.18	0.3522	0.11
12752	312.	0.5521	0.18	0.2091	0.07	1.5816	0.51	1.4839	0.48	0.7658	0.24	0.3913	0.12

ALL WEIGHTS WERE RECORDED IN GRAMS (G). ABS. WT. = ORGAN WEIGHT. REL. % TBW = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.

a. Rat 12741 was moribund sacrificed on day 32 of study.

b. Value was not recorded.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 19 (PAGE 2): TERMINAL BODY WEIGHTS, ORGAN WEIGHTS AND RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP I — CARRIER CONTROL — 0 PPM

RAT NUMBER	TERMINAL BODY WEIGHT	EPIDIDYMIS RIGHT		TESTIS RIGHT		PROSTATE VENTRAL		PITUITARY		LIVER		ADRENALS PAIRED	
		ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW a	ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW
12737	313.	0.4551	0.14	1.6698	0.53	0.2300	0.07	b	----	14.01	4.48	0.062	0.02
12738	304.	0.4884	0.16	1.7502	0.58	0.3300	0.11	0.007	2.30	12.31	4.04	0.065	0.02
12739	269.	0.4659	0.17	1.4583	0.54	0.2900	0.11	0.006	2.23	10.61	3.95	0.044	0.02
12740	314.	0.5549	0.18	1.6062	0.51	0.3000	0.10	0.011	3.50	11.35	3.61	0.066	0.02
12741c	----	b	----	b	----	------	----	-----	----	------	----	-----	----
12742	270.	0.6011	0.22	1.6883	0.62	0.2800	0.10	0.015	5.55	10.26	3.80	0.080	0.03
12743	264.	0.5764	0.22	1.5546	0.59	0.2500	0.09	0.006	2.27	10.94	4.14	0.079	0.03
12744	343.	0.5055	0.15	1.5261	0.44	0.3700	0.11	0.008	2.33	13.80	4.02	0.061	0.02
12745	314.	0.5690	0.18	1.5403	0.49	b	----	0.006	1.91	12.05	3.83	0.070	0.02
12746	268.	0.6143	0.23	1.4721	0.55	0.3600	0.13	0.005	1.87	9.29	3.47	0.051	0.02
12747	316.	0.5917	0.19	1.8315	0.58	0.2600	0.08	0.009	2.85	11.49	3.64	0.062	0.02
12748	340.	0.5464	0.16	1.7023	0.50	0.3777	0.11	0.012	3.53	12.73	3.75	0.070	0.02
12749	237.	0.4711	0.20	1.4211	0.60	0.2609	0.11	0.008	3.38	9.92	4.19	0.060	0.02
12750	340.	0.5192	0.15	1.7108	0.50	0.4276	0.12	0.011	3.24	14.55	4.28	0.072	0.02
12751	310.	0.5133	0.16	1.5280	0.49	0.2561	0.08	0.006	1.94	12.82	4.14	0.050	0.02
12752	312.	0.5144	0.16	1.5763	0.50	0.4958	0.16	0.009	2.88	12.83	4.11	0.065	0.02

ALL WEIGHTS WERE RECORDED IN GRAMS (G). ABS. WT. = ORGAN WEIGHT. REL. % TBW = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.

a. Value was multiplied by 1000.
b. Value was not recorded.
c. Rat 12741 was moribund sacrificed on day 32 of study.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 19 (PAGE 3): TERMINAL BODY WEIGHTS, ORGAN WEIGHTS AND RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP I — CARRIER CONTROL — 0 PPM

RAT NUMBER	TERMINAL BODY WEIGHT	THYROID ABS. WT.	THYROID REL. % TBW a	PROSTATE DORSAL ABS. WT.	PROSTATE DORSAL REL. % TBW
12737	313.	0.014	4.48	0.2700	0.09
12738	304.	0.011	3.61	0.3200	0.10
12739	269.	0.012	4.47	0.4500	0.17
12740	314.	0.032	10.19	0.3900	0.12
12741b	----	-----	-----	------	----
12742	270.	0.043	15.91	0.1700	0.06
12743	264.	0.028	10.59	0.2800	0.10
12744	343.	0.027	7.87	0.3800	0.11
12745	314.	0.018	5.72	0.4400	0.14
12746	268.	0.019	7.10	0.2600	0.10
12747	316.	0.025	7.92	0.3700	0.12
12748	340.	0.029	8.54	0.3953	0.12
12749	237.	0.016	6.76	0.1991	0.08
12750	340.	0.038	11.18	0.3722	0.11
12751	310.	0.032	10.32	0.4486	0.14
12752	312.	0.021	6.73	0.3738	0.12

ALL WEIGHTS WERE RECORDED IN GRAMS (G). ABS. WT. = ORGAN WEIGHT. REL. % TBW = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.

a. Value was multiplied by 1000.

b. Rat 12741 was moribund sacrificed on day 32 of study.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 19 (PAGE 4): TERMINAL BODY WEIGHTS, ORGAN WEIGHTS AND RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP II — LOW DOSAGE — 100 PPM

RAT NUMBER	TERMINAL BODY WEIGHT	EPIDIDYMIS LEFT ABS. WT.	EPIDIDYMIS LEFT REL. % TBW	CAUDA EPIDIDYMIS LEFT ABS. WT.	CAUDA EPIDIDYMIS LEFT REL. % TBW	TESTIS LEFT ABS. WT.	TESTIS LEFT REL. % TBW	L. TESTIS MINUS TUNICA ALBUGINEA ABS. WT.	L. TESTIS MINUS TUNICA ALBUGINEA REL. % TBW	SEMINAL VESICLES WITH FLUID ABS. WT.	SEMINAL VESICLES WITH FLUID REL. % TBW	SEMINAL VESICLES WITHOUT FLUID ABS. WT.	SEMINAL VESICLES WITHOUT FLUID REL. % TBW
12753a	----	b	----	------	----	c	----	------	----	------	----	------	----
12754	360.	0.5521	0.15	0.2075	0.06	1.8251	0.51	1.6020	0.44	0.8868	0.25	0.5200	0.14
12755	255.	0.4321	0.17	0.1576	0.06	1.4297	0.56	1.2952	0.51	0.8083	0.32	0.4037	0.16
12756	284.	0.5421	0.19	0.1969	0.07	1.6311	0.57	1.5224	0.54	0.7867	0.28	0.4574	0.16
12757	257.	0.4847	0.19	0.2078	0.08	1.6397	0.64	1.5142	0.59	0.6983	0.27	0.3970	0.15
12758	289.	0.4816	0.17	0.1711	0.06	1.6734	0.58	1.5775	0.55	0.7094	0.24	0.4546	0.16
12759	298.	0.5363	0.18	0.1918	0.06	1.6943	0.57	1.6081	0.54	0.8291	0.28	0.5176	0.17
12760	311.	0.4847	0.16	0.1614	0.05	1.3916	0.45	1.2742	0.41	0.8021	0.26	0.4336	0.14
12761	267.	0.5087	0.19	0.1823	0.07	1.6338	0.61	1.4400	0.54	0.7037	0.26	0.4227	0.16
12762	266.	0.4781	0.18	0.1782	0.07	1.5578	0.59	1.4193	0.53	0.6786	0.26	0.4041	0.15
12763	322.	0.6536	0.20	0.2499	0.08	1.6653	0.52	1.5336	0.48	0.5260	0.16	0.2941	0.09
12764	332.	0.5717	0.17	0.2084	0.06	1.8155	0.55	1.6510	0.50	0.9413	0.28	0.5282	0.16
12765	313.	0.6610	0.21	0.3199	0.10	1.8100	0.58	1.6747	0.54	0.9752	0.31	0.5106	0.16
12766	265.	0.4610	0.17	0.1794	0.07	1.3736	0.52	1.2575	0.47	0.7504	0.28	0.3971	0.15
12767	324.	0.5658	0.17	0.2312	0.07	1.7714	0.55	1.6428	0.51	0.9851	0.30	0.6098	0.19
12768	271.	0.5073	0.19	0.2075	0.08	1.5675	0.58	1.4683	0.54	0.8033	0.30	0.4843	0.18

ALL WEIGHTS WERE RECORDED IN GRAMS (G). ABS. WT. = ORGAN WEIGHT. REL. % TBW = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.

a. Rat 12753 was moribund sacrificed on day 44 of study.
b. Paired weight (0.74 g).
c. Paired weight (3.10 g).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 19 (PAGE 5): TERMINAL BODY WEIGHTS, ORGAN WEIGHTS AND RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP II — LOW DOSAGE — 100 PPM

RAT NUMBER	TERMINAL BODY WEIGHT	EPIDIDYMIS RIGHT ABS. WT.	EPIDIDYMIS RIGHT REL. % TBW	TESTIS RIGHT ABS. WT.	TESTIS RIGHT REL. % TBW	PROSTATE VENTRAL ABS. WT.	PROSTATE VENTRAL REL. % TBW	PITUITARY ABS. WT.	PITUITARY REL. % TBW a	LIVER ABS. WT.	LIVER REL. % TBW	ADRENALS PAIRED ABS. WT.	ADRENALS PAIRED REL. % TBW
12753b	----	c	----	d	----	------	----	-----	----	------	----	-----	----
12754	360.	0.5590	0.16	1.7814	0.49	0.3300	0.09	0.004	1.11	14.11	3.92	0.072	0.02
12755	255.	0.4568	0.18	1.3931	0.54	0.3400	0.13	0.006	2.35	10.21	4.00	0.058	0.02
12756	284.	0.5570	0.20	1.7409	0.61	0.3100	0.11	0.007	2.46	10.38	3.65	0.094	0.03
12757	257.	0.5309	0.21	1.6044	0.62	0.3200	0.12	0.008	3.11	9.88	3.84	0.120	0.05
12758	289.	0.5116	0.18	1.7153	0.59	0.3000	0.10	0.012	4.15	11.52	3.99	0.092	0.03
12759	298.	0.6242	0.21	1.6844	0.56	0.2600	0.09	0.008	2.69	11.88	3.99	0.050	0.02
12760	311.	0.4799	0.15	1.3206	0.42	0.3100	0.10	0.008	2.57	11.98	3.85	0.070	0.02
12761	267.	0.5526	0.21	1.6604	0.62	0.2800	0.10	0.008	3.00	10.63	3.99	0.062	0.02
12762	266.	0.5644	0.21	1.6618	0.62	0.3600	0.14	0.007	2.64	9.79	3.69	0.060	0.02
12763	322.	0.5196	0.16	1.6784	0.52	0.2100	0.06	0.008	2.49	11.82	3.68	0.072	0.02
12764	332.	0.6895	0.21	1.8993	0.57	0.2400	0.07	0.008	2.41	13.30	4.01	0.065	0.02
12765	313.	0.5610	0.18	1.7230	0.55	0.3140	0.10	0.011	3.52	12.33	3.94	0.065	0.02
12766	265.	0.4230	0.16	1.4793	0.56	0.3377	0.13	0.008	3.02	9.61	3.63	0.043	0.02
12767	324.	0.5871	0.18	1.7722	0.55	0.3259	0.10	0.008	2.47	15.03	4.64	0.055	0.02
12768	271.	0.5014	0.18	1.5765	0.58	0.1958	0.07	0.014	5.16	11.91	4.39	0.063	0.02

ALL WEIGHTS WERE RECORDED IN GRAMS (G). ABS. WT. = ORGAN WEIGHT. REL. % TBW = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.

a. Value was multiplied by 1000.
b. Rat 12753 was moribund sacrificed on day 44 of study.
c. Paired weight (0.74 g).
d. Paired weight (3.10 g).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 19 (PAGE 6): TERMINAL BODY WEIGHTS, ORGAN WEIGHTS AND RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP II — LOW DOSAGE — 100 PPM

RAT NUMBER	TERMINAL BODY WEIGHT	THYROID ABS. WT.	THYROID REL. % TBW a	PROSTATE DORSAL ABS. WT.	PROSTATE DORSAL REL. % TBW
12753b	----	-----	-----	------	----
12754	360.	0.022	6.11	0.4300	0.12
12755	255.	0.015	5.88	0.2500	0.10
12756	284.	0.022	7.74	0.2900	0.10
12757	257.	0.017	6.62	0.2400	0.09
12758	289.	0.024	8.31	0.3200	0.11
12759	298.	0.019	6.38	0.2800	0.09
12760	311.	0.025	8.04	0.2800	0.09
12761	267.	0.026	9.76	0.3600	0.14
12762	266.	0.014	5.27	0.3600	0.14
12763	322.	0.022	6.84	0.3200	0.10
12764	332.	0.035c	10.55	0.3100	0.09
12765	313.	0.024	7.68	0.3901	0.12
12766	265.	0.023	8.68	0.3604	0.14
12767	324.	0.020	6.18	0.3005	0.09
12768	271.	0.018	6.63	0.4131	0.15

ALL WEIGHTS WERE RECORDED IN GRAMS (G). ABS. WT. = ORGAN WEIGHT. REL. % TBW = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.

a. Value was multiplied by 1000.
b. Rat 12753 was moribund sacrificed on day 44 of study.
c. Damaged during processing (weight not affected).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 19 (PAGE 7): TERMINAL BODY WEIGHTS, ORGAN WEIGHTS AND RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP III — MIDDLE DOSAGE — 1000 PPM

RAT NUMBER	TERMINAL BODY WEIGHT	EPIDIDYMIS LEFT		CAUDA EPIDIDYMIS LEFT		TESTIS LEFT		L. TESTIS MINUS TUNICA ALBUGINEA		SEMINAL VESICLES WITH FLUID		SEMINAL VESICLES WITHOUT FLUID	
		ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW
12769	328.	0.4649	0.14	0.1700	0.05	1.5961	0.49	1.4855	0.45	0.8042	0.24	0.4306	0.13
12770	312.	0.5217	0.17	0.1839	0.06	1.7579	0.56	1.6509	0.53	1.0310	0.33	0.5608	0.18
12771	333.	0.4863	0.15	0.2032	0.06	1.8788	0.56	1.6314	0.49	0.8417	0.25	0.4399	0.13
12772	353.	0.5804	0.16	0.2400	0.07	1.8091	0.51	1.6438	0.46	0.9095	0.26	0.5905	0.17
12773	351.	0.5641	0.16	0.2192	0.06	1.9288	0.55	1.7601	0.50	1.0533	0.30	0.5288	0.15
12774	276.	0.4943	0.18	0.1798	0.06	1.4473	0.52	1.3411	0.48	1.1115	0.40	0.5603	0.20
12775	306.	0.6050	0.20	0.2400	0.08	1.5757	0.51	1.4133	0.46	0.9078	0.30	0.4467	0.14
12776	336.	0.5728	0.17	0.1841	0.05	1.6389	0.49	1.5430	0.46	0.8439	0.25	0.4617	0.14
12777	349.	0.5796	0.16	0.2227	0.06	1.8076	0.52	1.6787	0.48	0.6426	0.18	0.4430	0.13
12778	254.	0.5061	0.20	0.1665	0.06	1.4096	0.56	1.2713	0.50	0.7775	0.31	0.4883	0.19
12779	305.	0.4962	0.16	0.1724	0.06	1.6651	0.54	1.5370	0.50	0.5035	0.16	0.2797	0.09
12780	326.	0.5509	0.17	0.2071	0.06	1.6821	0.52	1.5752	0.48	0.7778	0.24	0.5380	0.16
12781	274.	0.4566	0.17	0.1625	0.06	1.4384	0.52	1.3499	0.49	0.7616	0.28	0.3402	0.12
12782	279.	0.4958	0.18	0.2109	0.08	1.4882	0.53	1.3885	0.50	0.7546	0.27	0.4302	0.15
12783	351.	0.5748	0.16	0.2202	0.06	1.5956	0.45	1.4823	0.42	0.8324	0.24	0.4822	0.14
12784	267.	0.5259	0.20	0.2134	0.08	1.7200	0.64	1.5949	0.60	0.7211	0.27	0.4401	0.16

ALL WEIGHTS WERE RECORDED IN GRAMS (G). ABS. WT. = ORGAN WEIGHT. REL. % TBW = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 19 (PAGE 8): TERMINAL BODY WEIGHTS, ORGAN WEIGHTS AND RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP III — MIDDLE DOSAGE — 1000 PPM

RAT NUMBER	TERMINAL BODY WEIGHT	EPIDIDYMIS RIGHT		TESTIS RIGHT		PROSTATE VENTRAL		PITUITARY		LIVER		ADRENALS PAIRED	
		ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW a	ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW
12769	328.	0.4719	0.14	1.5889	0.48	0.3200	0.10	0.010	3.05	12.88	3.93	0.068	0.02
12770	312.	0.4870	0.16	1.7749	0.57	0.3300	0.10	0.010	3.20	11.16	3.57	0.066	0.02
12771	333.	0.4932	0.15	1.7549	0.53	0.2500	0.08	0.009	2.70	14.50	4.35	0.070	0.02
12772	353.	0.5760	0.16	1.8014	0.51	0.3400	0.10	0.004	1.13	13.56	3.84	0.081	0.02
12773	351.	0.5284	0.15	1.8509	0.53	0.3200	0.09	0.009	2.57	14.11	4.02	0.074	0.02
12774	276.	0.4493	0.16	1.4573	0.53	0.4100	0.15	0.011	3.98	9.90	3.58	0.064	0.02
12775	306.	0.6147	0.20	1.5864	0.52	0.3500	0.11	0.012	3.92	12.80	4.18	0.086	0.03
12776	336.	0.5382	0.16	1.6134	0.48	0.3400	0.10	0.007	2.08	12.72	3.79	0.054	0.02
12777	349.	0.5686	0.16	1.7099	0.49	0.4400	0.13	0.008	2.29	13.11	3.75	0.051	0.01
12778	254.	0.5274	0.21	1.4541	0.57	0.2400	0.09	0.006	2.37	9.42	3.72	0.064	0.02
12779	305.	0.5662	0.18	1.6594	0.54	0.2100	0.07	0.005	1.64	11.44	3.74	0.062	0.02
12780	326.	0.5144	0.16	1.6428	0.50	0.2548	0.08	0.009	2.76	12.95	3.97	0.062	0.02
12781	274.	0.4395	0.16	1.4014	0.51	0.2775	0.10	0.009	3.28	11.02	4.02	0.039	0.01
12782	279.	0.4771	0.17	1.5196	0.54	0.3209	0.11	0.010	3.58	10.75	3.85	0.059	0.02
12783	351.	0.6271	0.18	1.6367	0.46	0.3420	0.10	0.010	2.85	14.69	4.18	0.071	0.02
12784	267.	0.4878	0.18	1.6294	0.61	0.3709	0.14	0.009	3.37	11.83	4.43	0.056	0.02

ALL WEIGHTS WERE RECORDED IN GRAMS (G). ABS. WT. = ORGAN WEIGHT. REL. % TBW = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.
a. Value was multiplied by 1000.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 19 (PAGE 9): TERMINAL BODY WEIGHTS, ORGAN WEIGHTS AND RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP III MIDDLE DOSAGE 1000 PPM

RAT NUMBER	TERMINAL BODY WEIGHT	THYROID ABS. WT.	THYROID REL. % TBW a	PROSTATE DORSAL ABS. WT.	PROSTATE DORSAL REL. % TBW
12769	328.	0.032	9.76	0.3300	0.10
12770	312.	0.026	8.32	0.2900	0.09
12771	333.	0.015	4.50	0.2400	0.07
12772	353.	0.013	3.68	0.3100	0.09
12773	351.	0.023	6.56	0.3000	0.08
12774	276.	0.021	7.60	0.3400	0.12
12775	306.	0.034	11.10	0.5400	0.18
12776	336.	0.022	6.56	0.5200	0.15
12777	349.	0.031	8.88	0.6200	0.18
12778	254.	0.018	7.10	0.2500	0.10
12779	305.	0.022	7.20	0.4000	0.13
12780	326.	0.026	7.97	0.2562	0.08
12781	274.	0.030	10.93	0.3700	0.13
12782	279.	0.022	7.88	0.4403	0.16
12783	351.	0.029	8.26	0.5371	0.15
12784	267.	0.012	4.50	0.3056	0.11

ALL WEIGHTS WERE RECORDED IN GRAMS (G). ABS. WT. = ORGAN WEIGHT. REL. % TBW = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.
a. Value was multiplied by 1000.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 19 (PAGE 10): TERMINAL BODY WEIGHTS, ORGAN WEIGHTS AND RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP IV — HIGH DOSAGE — 10000 PPM

RAT NUMBER	TERMINAL BODY WEIGHT	EPIDIDYMIS LEFT ABS. WT.	EPIDIDYMIS LEFT REL. % TBW	CAUDA EPIDIDYMIS LEFT ABS. WT.	CAUDA EPIDIDYMIS LEFT REL. % TBW	TESTIS LEFT ABS. WT.	TESTIS LEFT REL. % TBW	L. TESTIS MINUS TUNICA ALBUGINEA ABS. WT.	L. TESTIS MINUS TUNICA ALBUGINEA REL. % TBW	SEMINAL VESICLES WITH FLUID ABS. WT.	SEMINAL VESICLES WITH FLUID REL. % TBW	SEMINAL VESICLES WITHOUT FLUID ABS. WT.	SEMINAL VESICLES WITHOUT FLUID REL. % TBW
12785	289.	0.4463	0.15	0.1698	0.06	1.6466	0.57	1.3188	0.46	0.2871	0.10	0.2476	0.08
12786	278.	0.4943	0.18	0.1684	0.06	1.7680	0.64	1.6342	0.59	0.6452	0.23	0.4342	0.16
12787	312.	0.5589	0.18	0.2498	0.08	1.5692	0.50	1.4000	0.45	1.1060	0.35	0.6304	0.20
12788	268.	0.4757	0.18	0.1886	0.07	1.4943	0.56	1.3334	0.50	0.9345	0.35	0.5156	0.19
12789	300.	0.5006	0.17	0.3179	0.10	1.6277	0.54	1.5105	0.50	0.6980	0.23	0.4598	0.15
12790	353.	0.6922	0.20	0.2136	0.06	1.7583	0.50	1.6151	0.46	0.9476	0.27	0.5088	0.14
12791	281.	0.5034	0.18	0.1699	0.06	1.7150	0.61	1.6074	0.57	0.8415	0.30	0.3926	0.14
12792	340.	0.7594	0.22	0.2403	0.07	1.7175	0.50	1.5601	0.46	0.8706	0.26	0.3876	0.11
12793	308.	0.5917	0.19	0.2290	0.07	1.6533	0.54	1.5212	0.49	0.8142	0.26	0.4499	0.14
12794	300.	0.6285	0.21	0.2316	0.08	1.6690	0.56	1.5401	0.51	1.0081	0.34	0.5236	0.17
12795	313.	0.5643	0.18	0.1951	0.06	1.7563	0.56	1.6439	0.52	0.6829	0.22	0.4127	0.13
12796	342.	0.5833	0.17	0.2156	0.06	1.6430	0.48	1.5550	0.45	0.8907	0.26	0.4632	0.14
12797	271.	0.4686	0.17	0.1643	0.06	1.4548	0.54	1.3380	0.49	0.8033	0.30	0.4874	0.18
12798	235.	0.5281	0.22	0.1707	0.07	1.3359	0.57	1.2494	0.53	0.7249	0.31	0.4061	0.17
12799	334.	0.6819	0.20	0.2263	0.07	1.6389	0.49	1.5137	0.45	0.8859	0.26	0.5490	0.16
12800	298.	0.5413	0.18	0.1993	0.07	1.7150	0.58	1.5787	0.53	0.8796	0.30	0.4743	0.16

ALL WEIGHTS WERE RECORDED IN GRAMS (G). ABS. WT. = ORGAN WEIGHT. REL. % TBW = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 19 (PAGE 11): TERMINAL BODY WEIGHTS, ORGAN WEIGHTS AND RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP IV — HIGH DOSAGE — 10000 PPM

RAT NUMBER	TERMINAL BODY WEIGHT	EPIDIDYMIS RIGHT		TESTIS RIGHT		PROSTATE VENTRAL		PITUITARY		LIVER		ADRENALS PAIRED	
		ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW a	ABS. WT.	REL. % TBW	ABS. WT.	REL. % TBW
12785	289.	0.4565	0.16	1.6173	0.56	0.2500	0.09	0.007	2.43	11.80	4.09	0.066	0.02
12786	278.	0.4784	0.17	1.6916	0.61	0.3000	0.11	0.010	3.60	10.49	3.77	0.072	0.02
12787	312.	0.5634	0.18	1.5146	0.48	0.5300	0.17	0.008	2.57	11.35	3.64	0.066	0.02
12788	268.	0.4310	0.16	1.5014	0.56	0.2500	0.09	0.007	2.61	9.20	3.43	0.064	0.02
12789	300.	0.4708	0.16	1.5909	0.53	0.3400	0.11	0.013	4.33	11.54	3.85	0.124	0.04
12790	353.	0.5991	0.17	1.8673	0.53	0.4200	0.12	0.008	2.27	15.62	4.43	0.063	0.02
12791	281.	0.5230	0.19	1.7579	0.63	0.4000	0.14	0.007	2.50	11.19	3.99	0.078	0.03
12792	340.	0.5579	0.16	1.5100	0.44	0.3400	0.10	0.006	1.76	15.11	4.44	0.069	0.02
12793	308.	0.5917	0.19	1.7367	0.56	0.3500	0.11	0.006	1.95	13.60	4.41	0.064	0.02
12794	300.	0.5612	0.19	1.7734	0.59	0.2800	0.09	0.006	2.00	11.85	3.95	0.059	0.02
12795	313.	0.5926	0.19	1.6883	0.54	0.2851	0.09	0.019	6.08	13.23	4.23	0.088	0.03
12796	342.	0.5920	0.17	1.6213	0.47	0.3984	0.12	0.011	3.21	13.55	3.96	0.067	0.02
12797	271.	1.4766	0.54	1.4756	0.54	0.3025	0.11	0.008	2.96	10.68	3.95	0.056	0.02
12798	235.	0.6114	0.26	1.3864	0.59	0.2765	0.12	0.007	2.97	9.55	4.06	0.053	0.02
12799	334.	0.5502	0.16	1.6896	0.50	0.1998	0.06	0.010	2.99	14.32	4.29	0.072	0.02
12800	298.	0.5159	0.17	1.6190	0.54	0.3144	0.10	0.009	3.02	12.49	4.20	0.059	0.02

ALL WEIGHTS WERE RECORDED IN GRAMS (G). ABS. WT. = ORGAN WEIGHT. REL. % TBW = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.
a. Value was multiplied by 1000.

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TABLE 19 (PAGE 12): TERMINAL BODY WEIGHTS, ORGAN WEIGHTS AND RATIOS (%) OF ORGAN WEIGHT TO TERMINAL BODY WEIGHT - INDIVIDUAL DATA - MALE RATS

DOSAGE GROUP IV HIGH DOSAGE 10000 PPM

RAT NUMBER	TERMINAL BODY WEIGHT	THYROID ABS. WT.	THYROID REL. % TBW a	PROSTATE DORSAL ABS. WT.	PROSTATE DORSAL REL. % TBW
12785	289.	0.018	6.24	0.2100	0.07
12786	278.	0.046	16.55	0.2200	0.08
12787	312.	0.015	4.81	0.3500	0.11
12788	268.	0.013	4.84	0.0100	0.00
12789	300.	0.043	14.33	0.2000	0.07
12790	353.	0.022	6.24	0.2600	0.07
12791	281.	0.044	15.69	0.2800	0.10
12792	340.	0.012	3.53	0.3600	0.10
12793	308.	0.026	8.43	0.3200	0.10
12794	300.	0.020	6.67	0.2700	0.09
12795	313.	0.024	7.68	0.3346	0.11
12796	342.	0.031b	9.06	0.4987	0.14
12797	271.	0.023	8.50	0.2811	0.10
12798	235.	0.029	12.32	0.4109	0.17
12799	334.	0.033	9.88	0.3790	0.11
12800	298.	0.036	12.10	0.2459	0.08

ALL WEIGHTS WERE RECORDED IN GRAMS (G). ABS. WT. = ORGAN WEIGHT. REL. % TBW = (ORGAN WEIGHT/TERMINAL BODY WEIGHT) X 100.

a. Value was multiplied by 1000.

b. Damaged during processing (weight not affected).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 20 (PAGE 1): SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - MALE RATS
(See footnotes at the end of this table.)

DOSAGE GROUP I	CARRIER CONTROL				0 PPM				
	VAS DEFERENS SPERM MOTILITY				CAUDA EPIDIDYMAL SPERM COUNT		TESTICULAR SPERMATID COUNT		
RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM DENSITY c	SPERMATID COUNT d	SPERMATID DENSITY e	DAILY SPERM PRODUCTION f
12737	316	95	15	331	69	1214.1	58	101.1	27.9
12738	248	94	16	264	51	762.8	62	105.7	29.5
12739	243	97	7	250	111	1588.0	48	106.7	23.0
12740	282	92	23	305	39	508.2	66	122.9	31.1
12741	MORIBUND SACRIFICED ON DAY 32 OF STUDY								
12742	240	96	10	250	45	558.2	81	146.6	38.5
12743	278	97	9	287	3	52.9	81	167.1	38.5
12744	228	98	5	233	50	576.7	66	136.2	31.1
12745	263	95	15	278	37	436.3	74	159.9	35.2
12746	343	97	9	352	71	1192.0	50	106.9	23.8
12747	341	94	22	363	69	788.6	53	95.2	25.4
12748	457	87	66	523	41	571.0	105	192.6	50.0
12749	484	94	30	514	25	391.3	75	166.7	36.1
12750	313	97	11	324	63	860.8	125	189.8	59.8
12751	266	89	33	299	29	446.0	108	228.9	51.6
12752	300	94	19	319	42	581.0	106	206.6	50.8

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TABLE 20 (PAGE 2): SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - MALE RATS
(See footnotes at the end of this table.)

DOSAGE GROUP II		LOW DOSAGE		100 PPM					
		VAS DEFERENS SPERM MOTILITY			CAUDA EPIDIDYMAL SPERM COUNT		TESTICULAR SPERMATID COUNT		
RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM DENSITY c	SPERMATID COUNT d	SPERMATID DENSITY e	DAILY SPERM PRODUCTION f
12753	MORIBUND SACRIFICED ON DAY 44 OF STUDY								
12754	588	93	41	629	47	655.2	68	122.8	32.8
12755	440	93	32	472	144	2643.1	60	134.0	28.7
12756	405	95	23	428	68	1248.1	51	96.9	24.6
12757	216	95	12	228	39	542.9	41	78.3	19.7
12758	108	91	11	119	53	896.0	87	159.5	41.8
12759	397	97	14	411	56	844.6	29	52.2	13.9
12760	187	94	13	200	37	663.1	44	99.9	21.3
12761	576	95	30	606	67	1063.1	34	68.3	16.4
12762	211	95	11	222	25	405.8	35	71.3	16.4
12763	359	91	36	395	62	717.7	49	92.4	23.8
12764	340	99	4	344	53	735.7	92	161.2	44.3
12765	206	89	25	231	330	2984.0	99	171.0	47.5
12766	375	95	20	395	96	1547.9	85	195.5	41.0
12767	366	93	27	393	75	938.4	120	211.3	57.4
12768	406	68	191	597	22	306.7	100	197.0	47.5

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TABLE 20 (PAGE 3): SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - MALE RATS
(See footnotes at the end of this table.)

DOSAGE GROUP III	MIDDLE DOSAGE			1000 PPM					
	VAS DEFERENS SPERM MOTILITY			CAUDA EPIDIDYMAL SPERM COUNT		TESTICULAR SPERMATID COUNT			
RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM DENSITY c	SPERMATID COUNT d	SPERMATID DENSITY e	DAILY SPERM PRODUCTION f
12769	193	83	39	232	35	595.6	43	83.7	20.5
12770	281	95	15	296	34	534.8	52	91.1	24.6
12771	330	95	19	349	86	1224.3	37	65.6	18.0
12772	224	93	17	241	129	1554.8	40	70.4	18.9
12773	299	89	36	335	87	1148.1	38	62.5	18.0
12774	223	97	6	229	22	353.9	56	120.8	27.0
12775	286	90	32	318	71	855.8	102	208.8	48.4
12776	333	89	41	374	43	675.6	72	135.0	34.4
12777	187	66	95	282	45	584.5	72	124.1	34.4
12778	286	94	17	303	46	799.2	95	216.2	45.1
12779	407	96	16	423	47	788.6	36	67.8	17.2
12780	196	82	43	239	82	1145.4	124	227.7	59.0
12781	236	93	17	253	23	409.4	82	175.7	39.3
12782	361	91	34	395	53	726.9	59	122.9	27.9
12783	357	94	24	381	48	630.6	84	163.9	40.2
12784	204	98	4	208	51	691.3	51	92.5	24.6

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TABLE 20 (PAGE 4): SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - MALE RATS
(See footnotes at the end of this table.)

DOSAGE GROUP IV	HIGH DOSAGE	10000 PPM							
	VAS DEFERENS SPERM MOTILITY				CAUDA EPIDIDYMAL SPERM COUNT		TESTICULAR SPERMATID COUNT		
RAT NUMBER	NUMBER MOTILE	MOTILE PERCENT	STATIC COUNT (NONMOTILE)	TOTAL COUNT a	SPERM COUNT b	SPERM DENSITY c	SPERMATID COUNT d	SPERMATID DENSITY e	DAILY SPERM PRODUCTION f
12785	306	91	30	336	85	1448.1	29	63.6	13.9
12786	248	96	9	257	53	910.4	86	152.2	41.0
12787	276	95	16	292	21	243.2	41	84.7	19.7
12788	475	91	49	524	56	858.9	31	67.3	14.8
12789	284	93	23	307	55	500.5	54	103.4	25.4
12790	205	93	15	220	42	568.8	85	152.2	41.0
12791	198	90	22	220	38	647.0	61	109.8	29.5
12792	266	84	49	315	38	457.4	116	215.1	55.7
12793	319	97	11	330	32	404.2	38	72.3	18.0
12794	367	98	8	375	75	936.8	56	105.2	27.0
12795	213	85	37	250	53	785.8	72	126.7	34.4
12796	476	88	63	539	39	523.3	128	238.1	61.5
12797	435	95	24	459	57	1003.6	51	110.3	24.6
12798	172	86	29	201	29	491.4	44	101.9	21.3
12799	331	94	23	354	62	792.5	83	158.6	39.3
12800	201	86	34	235	55	798.3	122	223.5	58.2

TABLE 20 (PAGE 5): SPERM MOTILITY, COUNT, DENSITY AND SPERMATID COUNT - INDIVIDUAL DATA - MALE RATS

FOOTNOTES:

a. Sum of number motile and static count. Groups of five fields were evaluated until a sperm count of at least 200 was achieved or 20 fields were evaluated.

b. Sperm count used in the calculation of sperm density. Ten fields were evaluated.

c. The sperm density was calculated by dividing the sperm count by the volume in the image area (34.3×10^{6} mL), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the sperm concentration. The calculated sperm concentration value (rounded to 1 decimal place) was multiplied by 50 (volume) and divided by the weight of the left cauda epididymis (see Table 18 for the weight of the left cauda epididymis) to obtain the sperm density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

d. Spermatid count used in the calculation of spermatid density. Ten fields were evaluated.

e. The spermatid density was calculated by dividing the spermatid count by the volume in the image area (34.3×10^{6} mL), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the spermatid concentration. The calculated spermatid concentration value (rounded to 1 decimal place) was multiplied by 50 (volume) and divided by the weight of the left testis minus tunica albuginea (see Table 18 for the weight of the left testis minus tunica albuginea) to obtain the spermatid density. The calculated value will vary by approximately 0.8% from the Computer Automated Sperm Analysis because the digital image evaluated is slightly smaller (4 pixels) than the actual field causing a slight underestimate of the actual volume and an overestimate of the concentration.

f. The daily sperm production was calculated by dividing the spermatid count by the volume in the image area (34.3×10^{6} mL), multiplying by 2 (dilution factor) and multiplying by 10^{-6} to obtain the spermatid concentration. The calculated spermatid concentration value (rounded to 1 decimal place) was multiplied by 50 (volume) and divided by 6.1 days (which is the transit time for spermatids).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 21 (PAGE 1): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - MALE RATS

RAT NUMBER	NORMAL	DETACHED HEAD	NO HEAD	BROKEN FLAGE-LLUM	PIN HEAD	TWO HEADS & TAILS	SHORT SPERM HEAD	BANANA	COILED FLAGEL-LUM	BENT FLAGEL-LUM	BENT FLAGEL-LUM TIP	OTHER	PERCENT ABNORMAL
DOSAGE GROUP I					CARRIER CONTROL				0 PPM				
12737	199	0	1	0	0	0	0	0	0	0	0	0	0.5
12738	200	0	0	0	0	0	0	0	0	0	0	0	0.0
12739	200	0	0	0	0	0	0	0	0	0	0	0	0.0
12740	200	0	0	0	0	0	0	0	0	0	0	0	0.0
12742	200	0	0	0	0	0	0	0	0	0	0	0	0.0
12743	199	1	0	0	0	0	0	0	0	0	0	0	0.5
12744	197	1	2	0	0	0	0	0	0	0	0	0	1.5
12745	197	1	2	0	0	0	0	0	0	0	0	0	1.5
12746	197	0	1	2	0	0	0	0	0	0	0	0	1.5
12747	197	3	0	0	0	0	0	0	0	0	0	0	1.5
12748	193	7	0	0	0	0	0	0	0	0	0	0	3.5
12749	196	1	2	1	0	0	0	0	0	0	0	0	2.0
12750	198	1	0	0	0	0	0	0	1	0	0	0	1.0
12751	198	1	0	1	0	0	0	0	0	0	0	0	1.0
12752	191	4	4	1	0	0	0	0	0	0	0	0	4.5

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 21 (PAGE 2): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - MALE RATS

RAT NUMBER	NORMAL	DETACHED HEAD	NO HEAD	BROKEN FLAGELLUM	PIN HEAD	TWO HEADS & TAILS	SHORT SPERM HEAD	BANANA	COILED FLAGELLUM	BENT FLAGELLUM	BENT FLAGELLUM TIP	OTHER	PERCENT ABNORMAL
DOSAGE GROUP II					LOW DOSAGE				100 PPM				
12754	190	4	6	0	0	0	0	0	0	0	0	0	5.0
12755	199	1	0	0	0	0	0	0	0	0	0	0	0.5
12756	194	3	2	0	0	0	0	0	1	0	0	0	3.0
12757	200	0	0	0	0	0	0	0	0	0	0	0	0.0
12758	198	1	1	0	0	0	0	0	0	0	0	0	1.0
12759	196	2	2	0	0	0	0	0	0	0	0	0	2.0
12760	198	0	2	0	0	0	0	0	0	0	0	0	1.0
12761	198	0	2	0	0	0	0	0	0	0	0	0	1.0
12762	195	3	1	1	0	0	0	0	0	0	0	0	2.5
12763	197	2	1	0	0	0	0	0	0	0	0	0	1.5
12764	198	1	1	0	0	0	0	0	0	0	0	0	1.0
12765	198	2	0	0	0	0	0	0	0	0	0	0	1.0
12766	198	2	0	0	0	0	0	0	0	0	0	0	1.0
12767	193	5	2	0	0	0	0	0	0	0	0	0	3.5
12768	194	4	2	0	0	0	0	0	0	0	0	0	3.0

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 21 (PAGE 3): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - MALE RATS

RAT NUMBER	NORMAL	DETACHED HEAD	NO HEAD	BROKEN FLAGE-LLUM	PIN HEAD	TWO HEADS & TAILS	SHORT SPERM HEAD	BANANA	COILED FLAGEL-LUM	BENT FLAGEL-LUM	BENT FLAGEL-LUM TIP	OTHER	PERCENT ABNORMAL
DOSAGE GROUP III				MIDDLE DOSAGE					1000 PPM				
12769	195	5	0	0	0	0	0	0	0	0	0	0	2.5
12770	198	1	0	1	0	0	0	0	0	0	0	0	1.0
12771	199	1	0	0	0	0	0	0	0	0	0	0	0.5
12772	198	1	1	0	0	0	0	0	0	0	0	0	1.0
12773	193	1	5	1	0	0	0	0	0	0	0	0	3.5
12774	195	1	3	0	0	0	0	0	1	0	0	0	2.5
12775	195	4	1	0	0	0	0	0	0	0	0	0	2.5
12776	198	1	1	0	0	0	0	0	0	0	0	0	1.0
12777	200	0	0	0	0	0	0	0	0	0	0	0	0.0
12778	197	0	2	0	0	0	0	0	1	0	0	0	1.5
12779	198	2	0	0	0	0	0	0	0	0	0	0	1.0
12780	199	1	0	0	0	0	0	0	0	0	0	0	0.5
12781	195	2	3	0	0	0	0	0	0	0	0	0	2.5
12782	195	2	3	0	0	0	0	0	0	0	0	0	2.5
12783	198	1	1	0	0	0	0	0	0	0	0	0	1.0
12784	199	1	0	0	0	0	0	0	0	0	0	0	0.5

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 21 (PAGE 4): CAUDA EPIDIDYMAL SPERM MORPHOLOGY - INDIVIDUAL DATA - MALE RATS

RAT NUMBER	NORMAL	DETACHED HEAD	NO HEAD	BROKEN FLAGE-LLUM	PIN HEAD	TWO HEADS & TAILS	SHORT SPERM HEAD	BANANA	COILED FLAGEL-LUM	BENT FLAGEL-LUM	BENT FLAGEL-LUM TIP	OTHER	PERCENT ABNORMAL
DOSAGE GROUP IV				HIGH DOSAGE					10000 PPM				
12785	195	1	4	0	0	0	0	0	0	0	0	0	2.5
12786	199	0	0	1	0	0	0	0	0	0	0	0	0.5
12787	195	4	1	0	0	0	0	0	0	0	0	0	2.5
12788	197	2	1	0	0	0	0	0	0	0	0	0	1.5
12789	199	0	1	0	0	0	0	0	0	0	0	0	0.5
12790	196	4	0	0	0	0	0	0	0	0	0	0	2.0
12791	199	1	0	0	0	0	0	0	0	0	0	0	0.5
12792	192	7	1	0	0	0	0	0	0	0	0	0	4.0
12793	197	3	0	0	0	0	0	0	0	0	0	0	1.5
12794	196	1	3	0	0	0	0	0	0	0	0	0	2.0
12795	199	0	1	0	0	0	0	0	0	0	0	0	0.5
12796	196	3	1	0	0	0	0	0	0	0	0	0	2.0
12797	198	1	1	0	0	0	0	0	0	0	0	0	1.0
12798	199	0	1	0	0	0	0	0	0	0	0	0	0.5
12799	193	6	1	0	0	0	0	0	0	0	0	0	3.5
12800	192	4	2	2	0	0	0	0	0	0	0	0	4.0

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TABLE 22 (PAGE 1): TESTOSTERONE LEVELS (NG/ML) - INDIVIDUAL DATA - MALE RATS

WEEK	3	5	7	9
RAT #	DOSAGE GROUP I	CARRIER CONTROL		0 PPM
12737	0.352	0.362	1.139	0.464
12738	0.384	0.458	2.188	0.805
12739	0.491	2.213	2.903	0.776
12740	0.483	1.581	1.677	1.400
12741	0.641	4.021	a	
12742	1.839	0.785	1.119	1.021
12743	0.539	0.352	0.909	1.499
12744	0.294	1.373	1.941	1.013
12745	0.424	1.583	1.127	0.927
12746	1.017	0.815	1.964	0.872
12747	0.200	0.444	0.828	1.370
12748	0.167	0.431	0.432	2.247
12749	0.192	0.653	1.480	0.871
12750	0.370	1.620	2.124	1.256
12751	0.332	3.554	0.828	1.072
12752	0.323	2.426	1.428	0.677
RAT #	DOSAGE GROUP II	LOW DOSAGE		100 PPM
12753	0.360	0.367	1.719	b
12754	0.381	1.226	2.480	0.743
12755	0.433	0.987	2.253	0.730
12756	0.319	0.830	1.553	0.680
12757	0.358	3.921	1.434	2.141
12758	0.445	1.492	5.043	1.319
12759	0.410	1.129	1.322	3.164
12760	0.274	0.397	3.661	0.760
12761	0.267	0.834	0.714	2.610
12762	0.430	1.309	6.171	1.127
12763	0.191	1.064	1.860	0.872
12764	0.267	0.462	0.487	1.115
12765	0.515	1.742	1.533	2.085
12766	0.303	1.493	1.474	1.422
12767	0.252	0.885	1.087	1.396
12768	0.416	1.845	1.604	2.107

WEEK = WEEK OF STUDY
a. Rat 12741 was moribund sacrificed on day 32 of study (week 5).
b. Rat 12753 was moribund sacrificed on day 44 of study (week 7).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 22 (PAGE 2): TESTOSTERONE LEVELS (NG/ML) - INDIVIDUAL DATA - MALE RATS

WEEK	3	5	7	9
RAT #	DOSAGE GROUP III	MIDDLE DOSAGE		1000 PPM
12769	0.324	0.625	0.924	0.390
12770	0.358	0.588	1.350	0.535
12771	0.308	0.650	0.769	1.322
12772	0.365	0.975	1.144	0.926
12773	0.252	2.310	1.805	1.170
12774	0.287	3.815	1.317	1.247
12775	0.150	0.366	1.173	0.741
12776	0.506	1.256	1.349	1.600
12777	0.492	0.471	1.106	0.501
12778	0.351	0.854	1.104	0.994
12779	0.274	0.419	2.249	2.792
12780	0.241	0.314	0.315	0.927
12781	0.194	0.973	2.090	1.051
12782	0.338	1.026	0.784	0.910
12783	0.471	2.624	1.131	0.473
12784	0.285	1.386	1.617	0.918
RAT #	DOSAGE GROUP IV	HIGH DOSAGE		10000 PPM
12785	0.261	0.732	1.604	1.999
12786	0.311	1.215	2.248	0.578
12787	0.307	1.136	1.880	2.957
12788	0.279	0.822	2.804	1.300
12789	0.120	1.517	3.377	2.963
12790	0.400	0.595	1.192	1.773
12791	0.234	0.318	1.164	1.986
12792	0.258	0.860	1.699	3.568
12793	0.149	1.220	3.408	0.686
12794	0.207	0.789	0.943	0.876
12795	0.324	0.590	0.380	0.465
12796	0.386	1.574	2.565	1.513
12797	0.233	0.305	2.296	3.032
12798	0.246	0.423	3.925	1.989
12799	0.197	2.214	2.557	1.384
12800	0.205	0.437	2.744	2.689

WEEK = WEEK OF STUDY

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 23 (PAGE 1): FOLLICLE STIMULATING HORMONE LEVELS (NG/ML) - INDIVIDUAL DATA - MALE RATS

WEEK	3	5	7	9
RAT #	DOSAGE GROUP I	CARRIER CONTROL		0 PPM
12737	100.446	105.549	122.704	124.473
12738	122.198	99.715	125.258	129.220
12739	92.774	171.887	119.595	133.133
12740	140.498	164.536	101.076	134.918
12741	113.399	76.648	a	
12742	154.403	75.046	110.274	208.825
12743	116.492	88.157	91.676	b
12744	102.968	105.310	91.119	247.472
12745	129.141	111.732	107.670	b
12746	119.094	111.544	204.181	169.479
12747	69.553	81.429	167.687	173.763
12748	66.498	87.701	142.810	92.058
12749	68.885	61.015	169.961	94.448
12750	85.632	82.911	138.965	64.404
12751	74.415	82.438	192.567	58.198
12752	93.445	97.678	169.913	56.769
RAT #	DOSAGE GROUP II	LOW DOSAGE		100 PPM
12753	82.745	92.164	84.769	c
12754	76.728	90.790	92.031	113.358
12755	97.024	129.995	83.059	165.378
12756	113.033	109.536	89.456	239.524
12757	120.216	82.858	116.026	116.981
12758	115.597	79.415	92.585	266.472
12759	107.630	95.005	85.386	248.126
12760	110.715	111.888	87.651	243.882
12761	116.092	123.223	153.418	94.961
12762	115.651	61.952	165.618	94.961
12763	78.667	41.997	91.990	253.973
12764	62.053	57.157	154.930	77.801
12765	90.748	68.897	197.780	87.783
12766	118.831	104.544	224.480	71.730
12767	96.267	91.120	195.941	60.684
12768	96.383	99.064	165.327	66.467

WEEK = WEEK OF STUDY
a. Rat 12741 was moribund sacrificed on day 32 of study (week 5).
b. Sample was beyond the validated range; value excluded from group averages.
c. Rat 12753 was moribund sacrificed on day 44 of study (week 7).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 23 (PAGE 2): FOLLICLE STIMULATING HORMONE LEVELS (NG/ML) - INDIVIDUAL DATA - MALE RATS

WEEK	3	5	7	9
RAT #	DOSAGE GROUP III	MIDDLE DOSAGE		1000 PPM
12769	97.866	101.451	75.037	103.638
12770	149.185	178.068	110.741	163.336
12771	128.996	122.765	82.606	216.482
12772	120.886	144.548	94.121	236.528
12773	102.354	94.690	88.747	265.702
12774	112.842	130.798	136.611	109.175
12775	64.473	115.304	99.133	102.927
12776	76.783	108.767	94.191	92.091
12777	102.739	49.196	142.557	221.719
12778	114.768	92.282	237.839	64.661
12779	84.325	64.775	220.761	59.978
12780	103.615	98.972	94.588	70.065
12781	96.681	110.795	149.589	75.094
12782	141.963	86.395	218.091	128.462
12783	108.429	123.995	165.313	65.909
12784	73.046	110.682	223.823	113.910
RAT #	DOSAGE GROUP IV	HIGH DOSAGE		10000 PPM
12785	104.274	109.730	80.728	116.896
12786	99.856	140.463	96.378	150.794
12787	106.310	142.839	103.106	308.872
12788	95.470	124.070	85.395	180.296
12789	69.582	91.203	76.922	266.262
12790	78.935	75.904	71.700	326.161
12791	118.899	55.327	96.290	227.187
12792	94.751	107.725	133.325	329.480
12793	125.222	61.790	128.466	239.105
12794	117.846	69.476	118.853	265.177
12795	125.322	71.376	86.161	152.163
12796	119.160	73.623	75.125	189.233
12797	71.179	111.137	158.735	124.763
12798	74.414	96.530	156.585	102.779
12799	102.654	114.658	171.012	108.598
12800	72.119	84.211	143.261	91.427

WEEK = WEEK OF STUDY

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 24 (PAGE 1): LUTENIZING HORMONE LEVELS (NG/ML) - INDIVIDUAL DATA - MALE RATS

WEEK	3	5	7	9
RAT #	DOSAGE GROUP I	CARRIER CONTROL		0 PPM
12737	4.706	4.262	6.176	2.506
12738	4.841	5.256	8.858	2.742
12739	3.901	5.430	6.386	4.229
12740	5.642	6.623	7.178	4.004
12741	5.015	8.995	a	
12742	6.249	5.239	6.182	4.165
12743	5.540	6.670	7.117	5.484
12744	4.495	4.688	7.906	4.172
12745	4.984	8.229	6.521	4.601
12746	4.993	4.751	2.950	2.895
12747	2.641	5.543	3.316	3.069
12748	2.997	6.369	3.785	4.174
12749	2.442	3.870	3.813	4.512
12750	2.913	4.566	3.523	5.193
12751	2.784	7.155	4.240	4.667
12752	3.912	7.211	4.021	4.494
RAT #	DOSAGE GROUP II	LOW DOSAGE		100 PPM
12753	4.314	2.929	5.688	b
12754	4.519	2.836	5.234	2.528
12755	4.690	2.977	4.291	2.912
12756	5.233	3.175	4.764	3.002
12757	5.546	3.573	5.719	4.329
12758	5.333	3.578	4.914	3.510
12759	5.293	3.065	4.483	4.556
12760	4.133	3.248	5.122	3.283
12761	4.250	4.364	4.126	4.031
12762	4.709	3.972	3.193	3.741
12763	2.559	3.389	2.876	3.547
12764	2.256	3.207	3.413	4.882
12765	3.347	3.692	4.183	0.074
12766	2.370	3.803	3.786	4.412
12767	2.382	6.081	4.187	4.115
12768	3.012	7.313	4.058	4.386

WEEK = WEEK OF STUDY
a. Rat 12741 was moribund sacrificed on day 32 of study (week 5).
b. Rat 12753 was moribund sacrificed on day 44 of study (week 7).

PROTOCOL 1203-006: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

TABLE 24 (PAGE 2): LUTENIZING HORMONE LEVELS (NG/ML) - INDIVIDUAL DATA - MALE RATS

WEEK	3	5	7	9
RAT #	DOSAGE GROUP III	MIDDLE DOSAGE		1000 PPM
12769	4.267	3.965	7.247	3.053
12770	6.440	4.154	6.430	3.508
12771	4.532	3.603	5.854	3.305
12772	6.480	3.417	5.758	3.492
12773	5.421	4.081	5.317	3.490
12774	4.487	5.940	5.782	4.031
12775	3.468	3.806	6.018	3.131
12776	4.620	6.686	6.624	3.720
12777	4.605	6.100	3.472	3.254
12778	4.830	5.263	4.089	3.744
12779	2.327	4.403	3.433	5.288
12780	2.409	3.354	2.755	3.286
12781	2.001	3.599	3.303	3.839
12782	2.316	5.851	4.391	5.242
12783	2.305	9.815	3.585	3.075
12784	2.141	7.803	4.487	5.040
RAT #	DOSAGE GROUP IV	HIGH DOSAGE		10000 PPM
12785	3.587	2.502	5.616	3.294
12786	3.790	3.265	7.322	3.376
12787	4.459	3.315	9.294	3.053
12788	4.551	3.158	6.848	3.916
12789	3.567	4.682	6.018	3.983
12790	3.549	3.549	5.853	2.901
12791	5.558	3.368	7.490	3.032
12792	3.748	4.239	8.882	3.786
12793	3.856	3.434	3.213	3.389
12794	4.584	5.375	3.636	3.556
12795	2.705	3.619	2.626	2.813
12796	2.899	4.519	3.098	3.240
12797	1.756	4.229	3.514	5.341
12798	1.991	3.155	3.509	4.072
12799	3.020	7.780	4.250	4.484
12800	2.098	5.900	3.937	4.801

WEEK = WEEK OF STUDY

APPENDIX C

PROTOCOL

CHARLES RIVER
LABORATORIES
Discovery and Development Services
Argus Division

PROTOCOL 1203-006

STUDY TITLE

Oral (Diet) Reproduction Toxicity Study of Butylparaben in Male Rats

PURPOSE

The purpose of this study is to test for toxic effects/ disturbances resulting from oral (diet) exposure to butylparaben of Crl:(WI) BR male rats on spermatogenesis.

TESTING FACILITY

CR-DDS Argus Division
905 Sheehy Drive, Building A
Horsham, Pennsylvania 19044-1241
Telephone: (215) 443-8710
Telefax: (215) 443-8587

STUDY DIRECTOR

Alan M. Hoberman, Ph.D., DABT
Director of Research
Address as cited above for Testing Facility.
E-mail: alan.hoberman@argus.criver.com

SPONSOR

The Cosmetic, Toiletry and Fragrance Association (CTFA)
1101 17th Street NW
Suite 300
Washington, D.C. 20036

STUDY MONITOR

Linda Loretz, Ph.D., DABT
Telephone: (202) 331-1770
Telefax: (202) 331-1969
E-mail: loretzl@ctfa.org

REGULATORY CITATIONS

U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.

REGULATORY COMPLIANCE

This study will be conducted in compliance with the Good Laboratory Practice (GLP) regulations cited above.

All changes or revisions of this protocol shall be documented, signed by the Study Director and the Sponsor, dated and maintained with the protocol.

The Testing Facility's Quality Assurance Unit (QAU) will audit the protocol, the raw data and the report, and will inspect critical phases of those portions of the study conducted at the Testing Facility in accordance with the Standard Operating Procedures of the Testing Facility.

The final report will include a compliance statement signed by the Study Director that the report accurately reflects the raw data obtained during the performance of the study and that all applicable GLP regulations were followed in the conduct of the study. Should significant deviations from GLP regulations occur, each will be described in detail, together with how the deviation might affect the quality or integrity of the study.

Should any portion of the study be conducted by a subcontractor or by the Sponsor, the QAU for that facility will conduct critical phase inspections and audit respective results and reports for that study portion according to the SOPs of that facility. Such critical phase inspection reports and report audits will be submitted by that facility to the Study Director. The dates of the inspections and report submissions will be incorporated into a QAU Statement generated by that facility and provided to the Testing Facility for inclusion in the final report. In addition, that facility will provide a statement of GLP compliance, as described above, for inclusion in the final report. The archival location of any records generated by that facility will be identified in the final report.

STUDY SCHEDULE

See ATTACHMENT 1 to the protocol.

TEST ARTICLE AND CARRIER

Identification

Test Article

Butylparaben (Lot identification will be documented in the raw data)

The Sponsor will provide to the Testing Facility documentation or certification of the identity, composition, method of synthesis, strength and activity/purity of the test article. This documentation will be included in the final report.

Carrier

The meal form of CE-2 diet (CLEA Japan, Inc.). Results of feed analyses will be included in the raw data.

Neither the Sponsor nor the Study Director is aware of any potential contaminants likely to be present in the carrier that would interfere with the results of this study. Therefore, no analyses other than those mentioned in this protocol will be conducted.

Safety Precautions

Gloves, dust-mist/HEPA-filtered mask, appropriate eye protection and uniform/lab coat to be worn during diet preparation and exposure. The Material Safety Data Sheet (MSDS) is attached to the protocol (see ATTACHMENT 2).

Storage

Bulk Test Article:	Room temperature.
Carrier:	Room temperature.
Prepared Diets:	Room temperature.

All test article shipments to the Testing Facility should be addressed to the attention of Mark Coker, Manager of Formulation Laboratory, at the previously cited address and telephone number.

Shipments should include information concerning storage conditions and shipping cartons should be labeled appropriately. The recipient should be notified in advance of shipment.

FORMULATION

Frequency of Preparation

Formulations (diets) will be prepared at least once every two weeks at the Testing Facility.

Detailed preparation procedures are attached to this protocol (ATTACHMENT 3).

Adjustment for Activity/Purity

The test article will be considered 100% active/pure for the purpose of dosage calculations.

Testing Facility Reserve Samples

The Testing Facility will reserve a sample (approximately 1 g) of each lot of bulk test article and a sample (125 g) of each lot of the carrier used during the course of the study. Samples will be stored under the previously cited conditions.

ANALYSES

Results of required analyses will be provided to the Testing Facility for inclusion in the study report.

Samples additional to those described below may be taken if deemed necessary during the course of the study. Additional analyses, if required, will be documented by protocol amendment.

Before initiation of exposure, the homogeneity of test diets prepared at the concentrations bracketing the study concentrations will be verified using samples taken from a prestudy preparation. Results of the concentration analyses of the first test diet preparation to be used during the study will be approved by the Study Director prior to exposure.

Acceptance Criteria

Acceptance criteria for analytical results for each group are defined as follows: 1) Concentration results will be considered acceptable if the difference between the mean value found and the targeted concentration is $\leq 15\%$; 2) Homogeneity results will be considered acceptable if the relative standard deviation (RSD) of the mean value at each sampling location is $\leq 5\%$; and 3) Results of stability analysis are within $\pm$ 10% of the concentration found during the corresponding initial concentration analysis.

Bulk Test Article Sampling

A sample of approximately 1 g of the test article will be taken on the last day of exposure and sent (ambient conditions) for analysis. The recipient of this sample will be identified by protocol amendment. The recipient will be notified in advance of sample shipment.

Analyses of Prepared Formulations

Concentration and Homogeneity

Homogeneity of the prepared test diets will be verified prior to the initiation of exposure; concentration of the prepared diets will be verified prior to the initiation of exposure and during the course of the study.

Duplicate samples (25 g each) will be taken from the top, middle and bottom of each concentration to be used on study on the day of the prestudy preparation. One sample of each duplicate set will be shipped for analysis; the remaining samples will be retained at the Testing Facility as backup samples. Backup samples will be stored under the previously cited conditions and discarded at the Testing Facility following issue of the final report.

For each subsequent preparation, duplicate samples (25 g each) will be taken from the middle of the preparations for all concentrations on each day of preparation during the study. The analysis schedule of these diets will be based on the stability of the test article in the diet. For any diet analysis, one sample of each duplicate will be shipped for analysis; the remaining samples will be retained at the Testing Facility as backup samples. Backup samples will be stored under the previously cited conditions and discarded at the Testing Facility following issue of the final report.

Stability

Stability of the prepared formulations will be documented during this study in conjunction with a prestudy preparation. Stability will be determined for the lowest and highest concentrations to be used on study after storage at room temperature for at least two, four and eight weeks.

Triplicate samples (25 g each) will be taken from the lowest and highest concentrations on the day of the prestudy preparation. All stability samples will be shipped for analysis and stored at room temperature. At the scheduled timepoints after the initial analysis (homogeneity results obtained during the prestudy analyses will serve as time zero), duplicate samples will be analyzed. The remaining stability samples will serve as backups to be analyzed if the analytical results are not accepted from one of the stability analyses. The backup samples will be discarded once the stability results are accepted.

Analytical Laboratory

Samples to be analyzed will be shipped (ambient conditions) to:

Principal Investigator: Richard Norlin, M.S.
CR-DDS - Worcester Division
57 Union Street
Worcester, Massachusetts 01608
Telephone: (508) 890-0100
Telefax: (508) 753-1834
E-mail: dick.norlin@criver.com

The recipient will be notified in advance of sample shipment.

Diet Analysis

A sample (125 g each) of each lot of carrier used on study will be collected and shipped for analysis for phytoestrogen and paraben levels. The laboratory conducting the analysis of the diet samples will be added to the protocol by amendment. The recipient will be notified in advance of sample shipment.

DISPOSITION

Prepared diets will be discarded at the Testing Facility. Backup samples will be discarded at the Testing Facility following issue of the final report or at CR-DDS Worcester Division, Worcester, Massachusetts, upon acceptance of the analytical results. Disposition of the remaining bulk test article will be documented in the raw data.

TEST SYSTEM

Species/Strain and Reason for Selection

The Crl:(WI) BR rat was selected as the Test System because this strain of rat has been demonstrated to be sensitive to reproductive toxins and has been widely used throughout industry for reproductive toxicity evaluations.

Number

Fo Generation

Population acclimated	A total of 10 dams and their respective cross-fostered litters (sufficient number of rats to provide 8 litters of 8 male pups per litter).

F1 Generation

Population selected for study	64 male pups (16 per dosage group).

Body Weight and Age

Fo generation dams will be ordered to arrive at the Testing Facility on day 13, 14 or 15 postpartum. Actual body weights will be recorded the day of or day after receipt and will be documented in the raw data. The weight range will be included in the final report. (Day 1 postpartum is defined as the day of birth.)

Sex

Male rats will be evaluated.

Source

Charles River Laboratories, Inc.

The rats will be shipped in filtered cartons by air freight and/or truck from Charles River Laboratories, Inc., to the Testing Facility.

Identification

Fo Generation

Female rats are assigned temporary animal numbers at receipt. The rats will be permanently identified using Monel® self-piercing ear tags (Gey Band and Tag Co., Inc., No. MSPT 20101) or by tattoo according to the Standard Operating Procedures of the Testing Facility.

F1 Generation

On day 21 postpartum, pups assigned to study will be identified with Monel® self-piercing ear tags and/or by tail tattoo according to the Standard Operating Procedures of the Testing Facility.

ANIMAL HUSBANDRY

All cage sizes and housing conditions are in compliance with the *Guide for the Care and Use of Laboratory Animals*[1].

Housing

Fo Generation Rats/F1 Generation Litters

Each dam and delivered litter will be housed in a common nesting box during the postpartum period.

F1 Generation Rats

After weaning, the F1 generation rats will be individually housed in stainless steel wire-bottomed caging.

Nesting Material

Nesting material (bed-o'cobs®) will be provided.

Bedding will be changed as often as necessary to keep the animals dry and clean. Bedding changes will be documented in the raw data. Analyses for possible contamination are conducted annually and documented in the raw data.

Room Air, Temperature and Humidity

The animal room is independently supplied with at least ten changes per hour of 100% fresh air that has been passed through 99.97% HEPA filters. Room temperature will be maintained at 64°F to 79°F (18°C to 26°C) and monitored constantly. Room humidity will also be monitored constantly and maintained at 30% to 70%.

Light

An automatically controlled 12-hour light:12-hour dark fluorescent light cycle will be maintained. Each dark period will begin at 1900 hours. The light cycle may be adjusted by the Study Director or designee if deemed necessary to accommodate scheduled laboratory activities. Any such adjustment will be documented in the raw data.

Diet

Rats will be given either CE-2 diet (CLEA Japan, Inc.) only (carrier control group) or test diets prepared using CE-2 diet and the test article. These will be available *ad libitum* from individual feeders.

Water

Water will be available *ad libitum* from individual bottles attached to the cages or from an automatic watering access system. All water will be from a local source and passed through a reverse osmosis membrane before use. Chlorine will be added to the processed water as a bacteriostat; processed water is expected to contain no more than 1.2 ppm chlorine at the time of analysis. Water is analyzed monthly for possible bacterial contamination and twice annually for possible chemical contamination.

Contaminants

Neither the Sponsor nor the Study Director is aware of any potential contaminants likely to be present in the certified diet or in the drinking water at levels that would interfere with the results of this study. Therefore, no analyses other than those routinely performed by the feed supplier or those mentioned in this protocol will be conducted.

RANDOMIZATION

Fo Generation

The female rats will be naturally bred at the Supplier's facility by breeder male rats of the same source and strain. The day of delivery will be designated day 1 of lactation (postpartum). The female rats will be allowed to deliver their litters at the Supplier and shipped to arrive at the Testing Facility on days 13, 14 or 15 postpartum. Dams will be placed into nesting boxes in consecutive order, by day postpartum on the day of arrival.

F1 Generation

Day 1 of lactation (postpartum) is defined as the day of birth. On day 21 postpartum, 64 male pups will be selected for study using a computer-generated randomization procedure or a table of random units. Sixteen pups will be assigned to each exposure group.

A table of random units or a computer-generated randomization procedure will be used to select 6 male rats per group for histopathological evaluation of the liver, adrenal, thyroid and pituitary glands. These tissues from the remaining rats in each group will be frozen for possible hormone analysis.

ADMINISTRATION

Route and Reason for Choice

The oral route via the diet was selected for use because it is one possible route of human exposure.

Method and Frequency

A constant concentration of the test article in the diet will be offered to the male rats in each group, and the mg/kg/day dosages consumed will be calculated and presented for periods corresponding to body weight and feed consumption observations.

A carrier control and three test diet concentrations will be given to the rats. Rats (in Groups II-IV) will be given continual access to the test article in the diet for at least 56 days beginning on day 21 postpartum. Test diet concentrations may be adjusted if observed toxicity indicates that it is required.

Rationale for Dosage Selection

Dosages were selected by the Sponsor on the basis of previous studies conducted with the test article.

Dosage Levels and Concentrations

Dosage Group	Number of Rats	Concentration (ppm)	Argus Batch Number
I	16	0	B-1203-006-A(Day.Month.Year)
II	16	100	B-1203-006-B(Day.Month.Year)
III	16	1000	B-1203-006-C(Day.Month.Year)
IV	16	10000	B-1203-006-D(Day.Month.Year)

The test substance will be considered 100% pure for the purpose of dosage calculations.

TESTS, ANALYSES AND MEASUREMENTS - Fo GENERATION FEMALE RATS

Viability

Viability observations will be recorded at least twice daily.

Clinical Observations and/or General Appearance

Clinical observations will be recorded weekly. Maternal behavior will be recorded daily beginning the day after arrival at the Testing Facility.

Clinical observations may be recorded more frequently than cited above.

Body Weights

Body weights will be recorded weekly and before sacrifice (terminal weight).

Feed Consumption Values

Feed consumption will be monitored as feed is replenished on an as-needed basis.

METHOD OF SACRIFICE - Fo GENERATION FEMALE RATS

Fo generation rats will be sacrificed by carbon dioxide asphyxiation.

NECROPSY - Fo GENERATION FEMALE RATS

Fo generation rats will be discarded after sacrifice without further evaluation.

Scheduled Sacrifice of Dams with Litters Assigned to Study

On day 21 postpartum, Fo generation female rats with litters assigned to study will be sacrificed and discarded without further evaluation.

Scheduled Sacrifice of Dams with Litters Not Assigned to Study

Dams and litters not assigned to the study will be sacrificed after selection of pups for study assignment and it is determined that no additional pups will be needed. Carcasses will be discarded without further evaluation.

Dams with No Surviving Pups

Dams with no surviving pups will be sacrificed after the last pup is found dead or missing (presumed cannibalized). Carcasses will be discarded without further evaluation.

Rats Found Dead or Moribund

Rats that die or are sacrificed because of moribund condition will be discarded without further evaluation.

TESTS, ANALYSES AND MEASUREMENTS - F1 GENERATION MALE RATS

Viability

All Periods: At least twice daily.

Clinical Observations and/or General Appearance

Acclimation Period: Weekly.

Dosage Period: Once daily.

Clinical observations may be recorded more frequently than cited above.

Body Weights

Acclimation Period: Weekly.

Dosage Period: Daily.

Sacrifice: Terminal weight.

Feed Consumption Values (recorded and tabulated)

Dosage Period: Twice weekly.

Feed consumption values may be recorded more frequently than cited above if it is necessary to replenish the feed.

Blood Sample Collection for Hormone Levels

Beginning at the start of week 3 of the exposure period, blood samples (at least 1.6 mL each) will be collected bi-weekly (every other week) from each male rat assigned to study. The time of each blood collection will be recorded in the raw data. Blood samples will be collected at approximately the same time each week of collection (standardized for time of day) to address the circadian, pulsatile release of male hormones.

Blood will be collected from the orbital sinus. (If necessary, blood may be collected from an alternate site; if so, the alternate site will be documented in the raw data.) The rats will be anesthetized using isoflurane/oxygen before sample collection. The samples will be transferred into serum separator tubes and spun in a centrifuge. The resulting serum (0.8 mL) will be transferred into polypropylene tubes labeled with the protocol number, Sponsor study number, animal number, group number, dosage level, day of study, collection interval, date of collection, species, generation and storage conditions. All samples will be immediately frozen on dry ice and maintained frozen (approximately -80°C) until analysis at the Testing Facility. Samples will be analyzed for LH (luteinizing hormone), FSH (follicle-stimulating hormone) and testosterone.

Blood Sample Collection for Butylparaben and Para Hydroxy Benzoic Acid Levels

On the day of sacrifice, blood samples (at least 2 mL each) will be collected from each male rat assigned to study.

Blood will be collected from the vena cava after sacrifice. (If necessary, blood may be collected from an alternate site; if so, the alternate site will be documented in the raw data.) Blood tube identification, centrifugation and resulting aliquot will be added by protocol amendment. The polypropylene tubes will be labeled with the protocol number, animal number, group number, dosage level, day of study, collection interval, date of collection, species, generation and storage conditions. All samples will be immediately frozen on dry ice and maintained frozen (approximately -80°C) until shipment for butylparaben and para hydroxy benzoic acid level (metabolite) analysis.

Shipping Instructions

Shipping instructions will be added by protocol amendment.

METHOD OF SACRIFICE - F1 GENERATION MALE RATS

Rats will be sacrificed by carbon dioxide asphyxiation.

NECROPSY - F1 GENERATION MALE RATS

Gross lesions will be retained in neutral buffered 10% formalin for possible future evaluation (a table of random units will be used to select one control group rat from which all tissues examined at necropsy will be retained, in order to provide control tissues for any possible histopathological evaluations of gross lesions). Unless specifically cited below, all other tissues will be discarded.

Pups Found Dead or Moribund Before Exposure on Day 21 Postpartum (Day 1 of Study)

Pups that die or are sacrificed because of moribund condition before exposure will be discarded without further evaluation.

Pups Not Selected for Study

All pups not selected for study will be sacrificed on day 21 postpartum and discarded without further evaluation.

Scheduled Sacrifice and Sperm Evaluations

After completion of the exposure period, male rats will be sacrificed, and a gross necropsy of the thoracic, abdominal and pelvic viscera will be performed. To assess the potential toxicity of the test article on the male reproductive system, the endpoints listed below will be evaluated.

Organ Weights

The following organs will be individually weighed: liver, adrenal glands (paired), thyroid, pituitary, right testis, left testis, left epididymis (whole and cauda), right epididymis, seminal vesicles (with and without fluid) and prostate (ventral and dorsal). The left testis from each rat will be collected for Daily Sperm Production (DSP) determinations.

Frozen Tissue Archive

The liver, adrenal, thyroid and pituitary glands from 10 male rats per exposure group will be quick-frozen in liquid nitrogen and stored at approximately -80°C for possible hormone measurements.

Sperm Motility, Concentration, Morphology Evaluation and Daily Sperm Production

Sperm concentration and motility will be evaluated using computer-assisted sperm analysis (CASA). Motility will be evaluated by the Hamilton Thorne (Integrated Visual Optical System) IVOS by collection of a sample from the left vas deferens. A homogenate will be prepared from the left cauda epididymis for evaluation by the Hamilton Thorne IVOS to determine sperm concentration (sperm per gram of tissue weight). The remaining portion of the left cauda epididymis will be used to manually evaluate sperm morphology. Sperm morphology evaluations will include the following: 1) determination of the percentage of normal sperm in a sample of at least 200; and 2) qualitative evaluation of abnormal sperm, including such categories as abnormal head, abnormal tail, and abnormal head and tail.

The left testis will be used to evaluate DSP. The tunica will be removed, and the left testis will be reweighed. The tissue will be minced (using scissors) and homogenized using a Polytron homogenizer (1 minute in 50 mL 0.9% saline containing 0.05% Triton X-100). A sample of the homogenate (1 mL each) will be mixed with 0.5 mL of 4% trypan blue. The number of surviving spermatids in three 20 mcL aliquots will be counted using the Hamilton Thorne IVOS. The average number of spermatids per sample will be calculated. The total number of spermatids per testis and the number of spermatids per gram of testis will be calculated. DSP = spermatids per testis/6.1

Histopathology

The remaining portion of the left epididymis (corpus and caput), as well as the right epididymis, prostate and seminal vesicles will be fixed in neutral buffered 10% formalin for possible histopathological evaluation. The right testis will be fixed in modified Davidson's solution for 24 to 48 hours and then retained in neutral buffered 10% formalin for histopathological evaluation[2].

The liver, adrenal, thyroid and pituitary glands from six male rats per exposure group will be fixed in neutral buffered 10% formalin for histopathological evaluation.

Histological Evaluation

Tissues to be examined histologically will be routinely processed, embedded in paraffin, sectioned at 5 microns and stained with hematoxylin and eosin.

Histological examination of reproductive organs (remaining portions of the left epididymis, right epididymis, right testis, prostate and seminal vesicles) will be performed on all control and high test article concentration group rats. Additionally, the liver, adrenal, thyroid and pituitary glands from six rats in the control and high test article concentration groups will be evaluated. If lesions attributed to the test article are observed in the rats exposed to the high test article concentration, the same organs will be examined histologically in the rats exposed to the lower test article concentrations. If overt treatment-related changes to testes are observed by qualitative examination, no further histopathological evaluation of the testes will be required. If qualitative examination of testicular tissue does not reveal overt treatment-related effects, a detailed qualitative examination of the testes will be made, taking into account the tubular stages of the spermatogenic cycle. The examination will be conducted in order to identify treatment-related effects such as missing germ cell layers or types, retained spermatids, multinucleate or apoptotic germ cells and sloughing of spermatogenic cells into the lumen. Any cell- or stage-specificity of testicular findings will be noted[3].
Histopathological evaluations will be conducted by a Board Certified Veterinary Pathologist with expertise in reproductive pathology. Should results from the control and high dosage groups warrant examination of the lower dosage groups and conduct of quantitative evaluation, scheduled report dates will be adjusted accordingly. Additional costs will be incurred should these evaluations be required.

Shipping Instructions

The tissues will be shipped (ambient conditions) to:

Attention: Ms. Kristi Larson
EPL, Inc.
22866 Shaw Road
Sterling, Virginia 20166
Telephone: (703) 471-7060
Telefax: (703) 471-8447
E-mail: klarson@epl-inc.com

The recipient will be notified in advance of sample shipment.
Tissues for histopathological evaluation will be examined by Peter Mann, D.V.M., Diplomate, ACVP (Veterinary Pathologist).

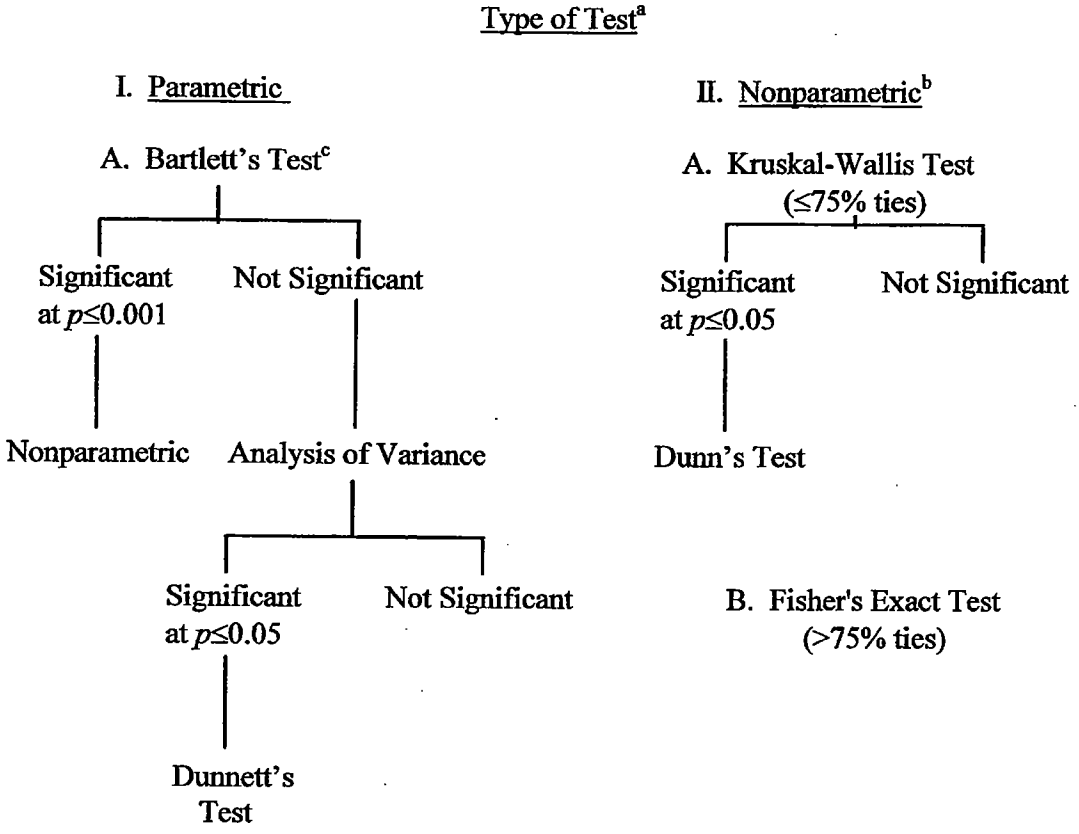
Rats Found Dead or Moribund During the Exposure Period

Rats that die or are sacrificed because of moribund condition will be examined for the cause of death or moribund condition on the day the observation is made. Gross necropsy will include an initial physical examination of external surfaces and all orifices, as well as an internal examination of tissues and organs *in situ*. In addition, the cranial, thoracic and abdominal cavities will be examined. Testes and epididymides will be excised and paired organ weights will be recorded. The lungs will be perfused with neutral buffered 10% formalin. Gross lesions will be retained in neutral buffered 10% formalin and examined histologically.

See ATTACHMENT 4 for tissues to be retained for possible histological evaluation (when not precluded by autolysis). All other tissues will be discarded.

PROPOSED STATISTICAL METHODS[4-10]

Averages and percentages will be calculated. Additional procedures and/or analyses may be performed, if appropriate.



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a. Statistically significant probabilities are reported as either $p \leq 0.05$ or $p \leq 0.01$.
b. Proportion data are not included in this category.
c. Test for homogeneity of variance.

DATA ACQUISITION, VERIFICATION AND STORAGE

Data generated during the course of this study will be recorded either by hand or using the *Argus Automated Data Collection and Management System*, the *Vivarium Temperature and Relative Humidity Monitoring System* and the *Hamilton Thorne IVOS*. All data will be tabulated, summarized and/or statistically analyzed using the *Argus Automated Data Collection and Management System*, the *Vivarium Temperature and Relative Humidity Monitoring System, Microsoft® Excel* (part of Microsoft® Office 97/2000/XP), Quattro Pro 8 and/or *The SAS System* (version 6.12).

Records will be reviewed by the Study Director and/or appropriate management personnel within 21 days after generation. Raw data (including slides) generated at EPL, Inc., will be sent to the Testing Facility. All original records will be stored in the archives of the Testing Facility. All original data will be bound and indexed. A copy of all raw data will be supplied to the Sponsor upon request. Preserved tissues will be stored at the Testing Facility at no additional charge for one year after mailing of the draft final report, after which time the Sponsor will be contacted to determine the disposition of these materials.

KEY PERSONNEL

Director of Research and Study Director: Alan M. Hoberman, Ph.D., DABT
Director of Operations: John F. Barnett, B.S.
Director of Study Management: Deanna L. Newcomb, B.S., LATG
Senior Manager, Regulatory Compliance: Matthew J. Vaneman, B.S.
Attending Veterinarian: Dena C. Lebo, V.M.D., Division Veterinarian
Chair, Institutional Animal Care and Use Committee: Douglas B. Learn, Ph.D.
Consultant, Veterinary Pathology: W. Ray Brown, D.V.M., Ph.D., Diplomate, ACVP
Consultant, Veterinary Pathology: Peter Mann, D.V.M., Diplomate, ACVP

RECORDS TO BE MAINTAINED

Protocol and Amendments.
Test Article, Vehicle and/or Reagent Receipt, Preparation and Use.
Animal Acquisition.
Randomization Schedules.
Treatment (if prescribed by Staff Veterinarian).
General Comments.
Clinical Observations and/or General Appearance.
Blood Sample Collection, Processing and Shipment.
Body Weights.
Feed Consumption Values.
Gross Necropsy Observations.
Sperm Evaluation:
- Sperm Motility.
- Sperm Count.
- Sperm Morphology.
- Sperm Staging.

Organ Weights.
Photographs (if required).
Study Maintenance (room and environmental records).
Feed and Water Analyses.
Packing and/or Shipment Lists.

FINAL REPORT

The Study Director will provide periodic updates of study progress to the Sponsor. Draft summary tables of unaudited computer-recorded data may accompany these updates. Statistical analyses will not be performed on these interim data.

A comprehensive draft final report will be prepared on completion of the study and will be finalized following consultation with the Sponsor. The report will include the following:

Summary and Conclusion.
Experimental Design and Method.
Evaluation of Test Results.
Appendices: Figures, Summary and Individual Tables Summarizing the Above Data, Protocol and Associated Amendments and Deviations, Study Director's GLP Compliance Statement, Reports of Supporting Data (if appropriate) and QAU Statement.

The Sponsor will receive one copy of the draft report. A copy of the final report will be provided on CD-ROM in Adobe Acrobat PDF format. The PDF document will be created from native electronic files to the extent possible, including text and tables generated by the Testing Facility. Report components not available in native electronic files and/or original signature pages will be scanned and converted to PDF image files for incorporation. A hard copy printed from the electronic file will accompany the final report on CD-ROM. The hard copy of the report with original signatures retained at the Testing Facility will be considered the GLP-compliant original.

Study reports should be finalized within six months of submission of the audited draft final report. Two Sponsor-requested revisions to the draft report will be addressed by the Testing Facility at no charge. Additional revisions to the draft report or amendments to the final report may incur additional costs. If the Sponsor has not provided comments to the report within six months of draft submission, the report will be finalized by the Testing Facility.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE STATEMENT

The procedures described in this protocol have been reviewed by the Testing Facility's Institutional Animal Care and Use Committee. All procedures described in this protocol that involve study animals will be conducted in a manner to avoid or minimize discomfort, distress or pain to the animals.

The signature of the Sponsor's representative below is assurance that the study is not an unnecessary duplication of previous work. Documentation for the necessity of this study may be obtained from the Sponsor. No alternative procedures were available to meet the stated purposes of the study.

REFERENCES

1. Institute of Laboratory Animal Resources (1996). *Guide for the Care and Use of Laboratory Animals*. National Academy Press, Washington, D.C.

2. Latendresse, J.R., Warbrittion, A.R., Jonassen, H. and Creasy, D.M. (2002). Fixation of testes and eyes using a modified Davidson's fluid: comparison with Bouin's fluid and conventional Davidson's fluid. Toxicologic Pathology 30(4): 524-533.

3. Lanning, L.L., Creasy, D.M., Chapin, R.E., Mann, P.C., Barlow, N.J., Regan, K.S. and Goodman, D.G. (2002). Recommended approaches for the evaluation of testicular and epididymal toxicity. Toxicologic Pathology 30(4): 507-520.

4. Snedecor, G.W. and Cochran, W.G. (1967). Variance test for homogeneity of the binomial distribution. *Statistical Methods*, 6th Edition, Iowa State University Press, Ames, pp. 240-241.

5. Sokal, R.R. and Rohlf, F.J. (1969). Bartlett's test of homogeneity of variances. *Biometry*, W.H. Freeman and Co., San Francisco, pp. 370-371.

6. Snedecor, G.W. and Cochran, W.G. (1967). Analysis of variance. *Statistical Methods*, 6th Edition, Iowa State University Press, Ames, pp. 258-275.

7. Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. J. Amer. Stat. Assoc. 50:1096-1121.

8. Sokal, R.R. and Rohlf, F.J. (1969). Kruskal-Wallis test. *Biometry*, W.H. Freeman and Co., San Francisco, pp. 388-389.

9. Dunn, O.J. (1964). Multiple comparisons using rank sums. Technometrics 6(3):241-252.

10. Siegel, S. (1956). The Fisher's exact probability test. *Nonparametric Statistics for the Behavioral Sciences.* McGraw-Hill Co., New York, pp. 96-105.

PROTOCOL APPROVAL

FOR THE TESTING FACILITY

for Raymond G. York, Ph.D., DABT
Associate Director of Research — Date: 10 June 2004

Alan M. Hoberman, Ph.D., DABT
Director of Research
Study Director — Date: 6-10-04

for Nancy A. Catricks, M.S.
Member, Institutional Animal Care and Use Committee — Date: 6-10-04

FOR THE SPONSOR

Linda Loretz, Ph.D., DABT
Study Monitor — Date: 6-28-04

ATTACHMENT 1

STUDY SCHEDULE

ATTACHMENT 1

STUDY SCHEDULE[a]

15 JUN 04	Fo Generation Female Rats and Litters Arrive - Acclimation Begins.
21 JUN 04 - 23 JUN 04	Exposure Begins - F1 Generation Pups [Day 21 postpartum (day 1 of study)].
21 JUN 04 - 23 JUN 04	Sacrifice - Fo Generation Female Rats (Day 21 postpartum).
16 AUG 04	End of Exposure Period (Earliest possible date)
18 AUG 04 - 20 AUG 04	Scheduled Sacrifice (Earliest possible dates).
17 NOV 04	Proposed Submission of Audited Draft Final Report.

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a. The start date of the study is the day the Study Director signs the protocol.

ATTACHMENT 2

MATERIAL SAFETY DATA SHEET

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MATERIAL SAFETY DATA SHEET

U.S. Department of Labor
Occupational Safety and Health Administration

SECTION I - MATERIAL AND MANUFACTURER IDENTIFICATION

Manufacturer's Name:	Emergency Telephone No.: 973-256-4374
Protameen Chemicals, Inc. 375 Minnisink Road Totowa, NJ 07511	
Date Prepared:	February 1, 1996
Common Name (used on label) (Trade Names & Synonyms):	Butyl Paraben
CAS No.:	94-26-8
Chemical Name:	Buthyl p-Hydroxy benzoate
Chemical Family:	Aromatic Carboxylic Acid
Formula:	$C_{11}H_{14}O_3$

SECTION II - HAZARDOUS INGREDIENTS/IDENTITY INFORMATION

PRINCIPAL HAZARDOUS COMPONENT(S) (CHEMICAL & COMMON NAME(S))	
% THRESHOLD LIMIT VALUE (UNITS)	NONE APPLICABLE

SECTION III - PHYSICAL & CHEMICAL CHARACTERISTICS (FIRE & EXPLOSION DATA)

BOILING POINT:	329°F
SPECIFIC GRAVITY (H_20=1):	1.05 (135°)
VAPOR PRESSURE (mm Hg):	0.5 mm/Hg (131°C)
PERCENT VOLATILE BY VOLUME (%):	NOT AVAILABLE
VAPOR DENSITY (AIR=1):	NOT AVAILABLE
EVAPORATION RATE:	NOT AVAILABLE
LUBILITY IN WATER:	0.03 g/100 ml (25°C)
REACTIVITY IN WATER:	NOT REACTIVE
APPEARANCE AND ODOR:	WHITE POWDER; ODORLESS OR A FAINT CHARACTERISTIC ODOR
FLASH POINT:	NOT AVAILABLE
FLAMMABLE LIMITS IN AIR	
% BY VOLUME:	UNKNOWN
LOWER:	-
UPPER:	-
EXTINGISHER MEDIA:	WATER, CO_2
AUTO IGNITION TEMPERATURE:	NOT AVAILABLE
SPECIAL FIRE FIGHTING PROCEDURES:	NO SPECIAL PROCEDURES ARE REQUIRED
UNUSUAL FIRE AND EXPLOSION HAZARDOUS:	NOTHING

SECTION IV - PHYSICAL DATA

STABILITY:	STABLE
CONDITIONS TO AVOID:	-
INCOMPATIBILITY(MATERIALS TO AVOID):	ALKALI, STRONG ACID
HAZARDOUS DECOMPOSITION PRODUCTS:	NOTHING
HAZARDOUS POLYMERIZATION:	WILL NOT OCCUR
CONDITIONS TO AVOID:	-

SECTION V - HEALTH HAZARDS

THRESHOLD LIMIT VALUE:	LD_{50}: 230 mg/Kg (ipr - mus)	
SIGNS & SYMPTOMS OF EXPOSURE:		
ACUTE OVEREXPOSURE:	NON IRRITATING TO THE SKIN	
CHRONIC OVEREXPOSURE:	NOT AVAILABLE	
MEDICAL CONDITIONS GENERALLY AGGRAVATED BY EXPOSURE:	-	
CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN:	-	
NATIONAL TOXICOLOGY PROGRAM:	I.A.R.C. MONOGRAPHS:	OSHA:
NO	NO	NO
OSHA PERMISSIBLE EXPOSURE LIMIT:	UNKNOWN	
ACGIH THRESHOLD LIMIT VALUE:	UNKNOWN	
OTHER EXPOSURE LIMIT USED:	UNKNOWN	

EMERGENCY AND FIRST AID PROCEDURES:

INHALATION:
REMOVE TO FRESH AIR. IF BREATHING IS DIFFICULT, GIVE OXYGEN. CALL A PHYSICIAN.

EYES:
IMMEDIATELY FLUSH EYES WITH PLENTY OF WATER AT LEAST 15 MIN. CALL A PHYSICIAN.

SKIN:
IMMEDIATELY WASH SKIN WITH SOAP AND PLENTY OF WATER.

INGESTION:
CALL A PHYSICIAN.

SECTION VI - SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION (SPECIFY TYPE):	MASK
VENTILATION:	
LOCAL EXHAUST:	REQUIRED
MECHANICAL(GENERAL):	-
SPECIAL:	-
OTHER:	-
PROTECTIVE GLOVES:	RUBBER GLOVES
EYE PROTECTION:	EYE GLASSES
OTHER PROTECTIVE CLOTHING OR EQUIPMENT:	NOTHING

SECTION VII - SPECIAL PRECAUTIONS AND SPILL/LEAK PROCEDURES

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE:	NO SPECIAL PRECAUTIONS ARE NECESSARY IN ITS HANDLING. STORED IN A DRY PLACE AND SHOULD NOT BE EXPOSED TO LIGHT OR HEAT.
OTHER PRECAUTIONS:	NOTHING
STEPS TO BE TAKEN IN CASE MATERIALS IS RELEASED OR SPILLED:	CLEAN UP BY VACUUM-CLEANER AND WASH BY WATER.
WASTE DISPOSAL METHODS:	ACTIVE SLUDGE METHOD OR BURNING OUT.

ATTACHMENT 3

TEST ARTICLE PREPARATION PROCEDURE

ATTACHMENT 3

TEST ARTICLE PREPARATION PROCEDURE

Test Article:	Butylparaben
Carrier:	The meal form of CE-2 Diet (CLEA Japan, Inc.)
Vehicle:	Acetone (used only for the purpose of transferring the test article to the carrier; the acetone dissipates and is not a permanent component of the diet mixture.)

A. Purpose:

The purpose of this procedure is to provide a method for the preparation of diet containing the test article for oral administration to rats on Argus Protocol 1203-006.

B. General Information:

1. All diet containers will be labeled and color coded. Each label will specify the protocol number, test article identification, Argus batch number, concentration, target dosage level, preparation date, expiration date and storage conditions.

2. Formulations (diets) will be prepared at least every two weeks at the Testing Facility.

3. See protocol for target concentrations.

4. Safety:
   - X Gloves, uniform/lab coat, goggles or safety glasses with side shields
   - X Dust-mist/HEPA-filtered Mask
   - ___ Half-Face Respirator
   - ___ Full-Face Respirator/Positive Pressure Hood
   - ___ Tyvek® Suit
   - ___ Bulk test article will be handled in a chemical fume hood

5. The test article will be considered 100% active/pure for the purpose of dosage calculations.

6. Sampling requirements: Cited in protocol

7. Storage: Cited in protocol

TEST ARTICLE PREPARATION PROCEDURE

C. Preparation of the Test Article for Addition to the Diet Mixtures:

1. Weigh out the required amount of test article (see PREPARATION CALCULATIONS) into an appropriately sized, labeled container.

2. Add approximately 20 mL of acetone to the container. Add a magnetic stir bar to the container, place on a magnetic stir plate and begin mixing. Add additional acetone to the mixture as necessary to dissolve the test article. This solution will be used in the preparation of the diet mixtures. NOTE: The amount of acetone added to each concentration, including the vehicle, will be same amount added to the test article for the high dose concentration. The actual amount of acetone used to dissolve the TA/S will be documented in the raw data.

3. Repeat steps C.1. and C.2. for each concentration.

D. Test Diet Preparation:

1. The following steps are completed for each concentration, beginning with the vehicle group preparation:

a. Weigh out the amount of certified rodent diet required to prepare a single concentration (see PREPARATION CALCULATIONS) into an appropriately sized, labeled container. All of the diet used in the subsequent procedure will be taken from this aliquot.

b. Check the placement of the intensifier bar in the twin shell blender then place approximately half of the diet into the twin shell blender.

c. Place approximately 3 kg of diet into the Hobart® mixing bowl.

ATTACHMENT 3

TEST ARTICLE PREPARATION PROCEDURE

d. Add the test article solution prepared above in section C to the Hobart® mixing bowl. Rinse the container several times with a total amount of an additional 20 mL of acetone. Add this rinse to the Hobart® mixing bowl as well.

e. Turn the Hobart® mixer on and mix for approximately 15 minutes. After mixing, transfer the feed/test article mixture from the Hobart® mixing bowl to the twin shell blender. Rinse the bowl by adding approximately 3 kg of diet from the aliquot to the bowl, return to the Hobart® mixer and mix for approximately 2 minutes. Add this "rinse" material to the twin shell blender.

f. Place the remaining diet from the aliquot into the twin shell blender, then close the twin shell blender. Turn on the twin shell blender, then turn on the intensifier bar. Check for leakage of feed from the lids.

g. Run the intensifier bar and blender for approximately fifteen minutes.

h. Following completion of the fifteen minutes, turn off the intensifier bar and blender.

i. Center the collection bag/container under the blender port and collect the prepared diet. Remove samples according to Testing Facility Standard Operating Procedures.

j. Repeat this process (steps D.1.a to D.1.j) for each concentration.

ATTACHMENT 3

TEST ARTICLE PREPARATION PROCEDURE

2. Clean the blender and intensifier bar according to Testing Facility Standard Operating Procedures.

Written By: Patricia A. Garbely

Approved By: [signature] Date: 10-JUN-04

Clarification: ___ No ✓ Yes (See attached clarification form.)

Initials/Date: TMZ 10/21/04

ARGUS

TEST ARTICLE/SUBSTANCE PREPARATION
PROCEDURE CLARIFICATION

Protocol: 1203-006 **Version:** 1203-006 (02 JUN 04)

Date of Clarification	Preparation Step #	Clarification
6/15/04	C.1	Test article may also be weighed into a weigh boat. If a weigh boat is needed, the test article should be transferred into an appropriately sized, labeled container. The test article should be transferred in small increments to aid in dissolution. MDB 6/15/04 C Hol 6/15/04 Study Director Date
6/15/04	C.2.	Note: Add the following amounts of acetone to the container to aid in dissolution of the test article: Group I 220mL, Group II 20mL, Group III 40mL, Group IV 220mL. Mix each container on a stir plate until a solution is observed. For Group IV to go into solution, add the test article slowly in small increments while adding ~~Study Director~~ (1) ~~Date~~ (1)
		the acetone volume in small increments while allowing the test article to remain a solution. MDB 6/15/04 C Hol 6/15/04 Study Director Date
		Study Director Date

Reviewed by: MDB 6/15/04 @MDB 6/28/04 Date: 6/15/04

08.15.97 TASPPM-01-02

(1)(A) MDB 6/15/04 MDB 6/28/04

ATTACHMENT 4

TISSUES TO BE RETAINED FOR POSSIBLE EXAMINATION

ATTACHMENT 4

TISSUES TO BE RETAINED FOR POSSIBLE EXAMINATION

The following tissues or representative samples will be collected at necropsy from rats found dead or sacrificed due to moribund condition and retained in neutral buffered 10% formalin.

Adrenals
Aorta
Bone marrow (sternum)
Brain (cerebrum, cerebellum, medulla/pons)
Epididymides
Esophagus
Eyes (with optic nerve)
Femur
Heart
Intestines, large (colon, cecum, rectum)
Intestines, small (duodenum, jejunum, ileum)
Kidneys
Liver
Lungs
Lymph nodes (mandibular, mesenteric)
Pancreas
Peyer's patches
Pituitary
Prostate
Salivary gland (mandibular)
Sciatic nerve
Seminal vesicles
Skeletal muscle
Skin
Spinal cord (cervical, mid-thoracic, lumbar)
Spleen
Stomach
Testes*
Thymus
Thyroid/Parathyroid
Trachea
Urinary Bladder

* The testes will be fixed in modified Davidson's solution for 24 to 48 hours and then retained in neutral buffered 10% formalin.

CHARLES RIVER
LABORATORIES
Discovery and Development Services
Argus Division

PROTOCOL 1203-006

ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

Amendment 1 - 17 September 2004

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1. Bulk Test Article Sampling (page 5 of the protocol):

[Effective Date: 17 August 2004] Analysis of the bulk test article will be conducted by Protameen Chemicals, Inc., Totowa, New Jersey, USA. The sample will be collected as described in the protocol and shipped (ambient conditions) to:

Principal Investigator: John J. Brodzinsky
Technical Director
Protameen Chemicals, Inc.
375 Minnisink Road
Totowa, New Jersey 07511
USA
Telephone: (973) 256-4374
Telefax: (973) 256-6764

The recipient will be notified in advance of sample shipment.

Reason for Change:

This information was to be added to the protocol by amendment.

Any revisions to this finalized amendment must be made by subsequent amendment.

2. Diet Analysis (page 6 of the protocol):

[Effective Date: 17 August 2004] Analysis of each lot of the carrier used on study for phytoestrogen and paraben levels will be conducted by Southern Testing and Research Labs, Wilson, North Carolina, USA. The sample will be collected as described in the protocol and shipped (ambient conditions) to:

Walter Hogg
Business Development and Client Services Manager
Southern Testing and Research Labs
A division of Microbac Laboratories, Inc.
3809 Airport Drive
Wilson, North Carolina 27896
USA
Telephone: (252) 237-4175
Telefax: (252) 237-9341

The recipient will be notified in advance of sample shipment.

Reason for Change:

This information was to be added to the protocol by amendment.

3. Blood Sample Collection for Butylparaben and Para Hydroxy Benzoic Acid Levels (page 14 of the protocol):

[Effective Date: 17 August 2004] The whole blood samples (approximately 2 mL each) will be transferred into EDTA-coated (purple top) tubes and spun in a centrifuge. The resulting plasma will be transferred into polypropylene tubes and labeled and processed as described in the final protocol. Samples will be maintained frozen (approximately -80°C) for possible future analysis.

Reason for Change:

This information was to be added to the protocol by amendment.

Any revisions to this finalized amendment must be made by subsequent amendment.

4. Sperm Motility, Concentration, Morphology Evaluation and Daily Sperm Production (page 16 of the protocol):

[Effective Date: 13 July 2004] Daily sperm production (testicular spermatid concentration) will be determined using the methods described below, rather than the method described in the final protocol.

The left testis will be used for evaluation of testicular spermatid concentration via CASA. The left testis will be weighed (both before and after removal of the tunica albuginea) and then homogenized. A sample from the resulting homogenate will be stained with an IDENT Stain Kit from Hamilton Thorne Research, and a slide will be prepared for analysis by the Hamilton Thorn IVOS. Ten fields will be analyzed to determine testicular spermatid concentration (spermatids per gram of tissue weight). All images produced during analysis will be retained as raw data.

The sperm concentration, motility and morphology evaluations will be conducted as described in the final protocol.

Reason for Change:

This change is being made with the Sponsor's agreement that the use of CASA to assist in testicular spermatid concentration determinations will not adversely impact re-evaluation of previously obtained results.

9-17-04
Raymond G. York, Ph.D., DABT Date
Associate Director of Research

17 Sep 04
Alan M. Hoberman, Ph.D., DABT Date
Director of Research
Study Director

17 SEP 2004
Douglas B. Learn, Ph.D. Date
Chair, Institutional Animal Care and Use Committee

9-27-04
Linda Loretz, Ph.D., DABT Date
Study Monitor

Any revisions to this finalized amendment must be made by subsequent amendment.

APPENDIX D

CERTIFICATE OF ANALYSIS

PROTAMEEN CHEMICALS INC.

375 Minnisink Road
Totowa, N.J. 07511
Office: 973-256-4374
Fax: 973-256-6764

Certificate of Analysis

Product Number: 330
Product Name: BUTYL PARABEN
Lot Number: B3140

Customer Name:
Customer PO#:
Customer Product Code:
Customer Prod Name:

Test Name:	Range:	Result:
Appearance @ 25dgr	White crystalline powder	White crystalline powder
Assay (%)	99.0 - 100.5	99.5
Identification - IR	Complies with standard	Pass
Melting Range Celcius	68 - 72	70
Acidity	Complies wiith standard	Conforms
on Drying %	0.5 Maximum	0.02
Residue on Ignition	0.05 Maximum	0.05
Organic Volatile Impurities	Complies with standard	Will Comply

ration Date: 2/10/2006 **Manufacture Date:** 2/11/2003

Issue Date: 10/14/2003 **Signature:** JOHN J. BRODZINSK
TECHNICAL DIRECTO

Remarks: MANUFACTURED BY UENO FINE CHEMICALS, OSAKA, JAPAN

APPENDIX E

ANALYTICAL REPORT AND FEED ANALYSES

CHARLES RIVER
LABORATORIES
Discovery and Development Services
Worcester Division

FINAL REPORT

METHOD VALIDATION AND FORMULATION SAMPLE ANALYSIS FOR CHARLES RIVER LABORATORIES DISCOVERY AND DEVELOPMENT SERVICES ARGUS DIVISION STUDY 1203-006

Project Number: PACA-ED00

Submitted to:

Charles River Laboratories
Discovery and Development Services
Argus Division
905 Sheehy Drive, Building A
Horsham, PA 19044-1241

Submitted by:

Charles River Laboratories
Discovery and Development Services
Worcester Division
57 Union Street
Worcester, MA 01608

Report No. PACA-ED00-04-896

Issue Date: March 17, 2005

Richard Norlin 3/17/05

Richard Norlin, M.S./Date
Principal Investigator
Charles River Laboratories
Discovery and Development Services
Worcester Division

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2. COMPLIANCE STATEMENT

This project was conducted in compliance with the U.S. Food and Drug Administration Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58 with the following exception:

1. The chromatographic data collection system, Hewlett-Packard ChemStation, has not been validated. This data collection system is on Charles River's Validation Schedule. The use of this non-validated chromatographic data did not affect the quality or integrity of the study or the interpretation of the results in this report.

2. The expiration dates for the internal standard, Isopropyl 4-Hydroxybenzoate, were not provided by the supplier.

Principal Investigator:

Richard Norlin 3/17/05

Richard Norlin, M.S./Date

3. QUALITY ASSURANCE STATEMENT

The following are the inspection dates and report dates of QAU audit/inspections for "Method Validation and Formulation Sample Analysis for Charles River Laboratories Discovery and Development Services Argus Division Study 1203-006", Protocol Number 1203-006; Charles River Laboratories Project Number PACA-ED00.

Critical Phases	Date Inspected	Date Report Submitted to Study Director	Date Report Submitted to Management
1. Laboratory Procedure	08/02/2004	11/8/2004	08/19/2004
2. Data	11/1,3,4/2004	11/8/2004	11/8/2004
3. Draft Final Report	11/1,3,4/2004	11/8/2004	11/8/2004

The Final Report for Charles River Laboratories Report Number PACA-ED00-04-896, was reviewed for compliance with the U.S Food and Drug Administration. Good Laboratory Practice Regulations, Final Rule. 21 CFR Part 58, on 03/17/2005. The results as presented accurately reflect the raw data.

Susan Caldbeck , 17/Mar/05

Susan Caldbeck Date
Quality Assurance Auditor

4. CONTRIBUTING PERSONNEL

Project Scientist .....Dorothy Savage, B.S.

Principal Investigator.....Richard Norlin, M.S.

Senior Director, Analytical Chemistry ..... Stephen Guyan, M.Sc.

Report Coordinator ..... Amanda Rappleyea, B.S.

5. ANALYTICAL REFERENCE STANDARD CHARACTERIZATION/STABILITY

Analytical Reference Standard:	Butylparaben
Physical Description:	White Solid
Storage Conditions:	22±5°C
Lot Number:	B3140
Date Received:	02-Apr-2004
Expiration/Retest Date:	10-Feb-2006
Amount Received:	2 g
Manufacturer/Supplier:	Charles River Laboratories Argus Division
Purity:	99.5%
Internal Reference Standard (Internal Standard):	Isopropyl 4-Hydroxybenzoate
Physical Description:	White Solid
Storage Conditions:	22±5°C
Lot Number:	C10H1203
Date Received:	21-Mar-2003 and 14-Jun-2004
Expiration/Retest Date:	Not Provided
Amount Received:	1 g
Manufacturer/Supplier:	Aldrich Chemical Co.
Purity:	Assumed 100%

5.1. Characterization and Stability

The characterization of the analytical reference standards is the responsibility of the Sponsor, as are the methods of synthesis, fabrication or derivation and stability determinations. The expiration date for the internal standard, Isopropyl 4-Hydroxybenzoate, was not provided by the supplier.

6. ARCHIVAL STORAGE

The original final report and raw data will be maintained for a minimum period of one year following submission of the final report in the Charles River Laboratories Discovery and Development Services Argus Division archives department located in Horsham, PA. After one year, storage disposition will be negotiated with the Sponsor. The Sponsor will be notified prior to disposal of any original study data. Archival material will be indexed by Report Number PACA-ED00-04-896.

7. ABSTRACT

Procedures were developed and validated for the analysis of Butylparaben in CE-2 Diet formulations. The procedures were adapted from a method provided by The Cosmetic, Toiletry and Fragrance Association and involve analysis of the compound by Gas Chromatography (GC) with Mass Spectrometry (MS) detection.

The procedures are applicable for the analysis of dose formulations at the following concentrations: 1.0 μg/g, 100 μg/g, 1000 μg/g and 10,000 μg/g of Butylparaben in CE-2 Diet. Validation of the Butylparaben formulation analysis was performed using a single-point calibration at a target concentration of 0.2 μg/mL. During method validation, the calibration standards were prepared at a concentration range spanning approximately 0.1 to 0.3 μg/mL of butylparaben in diluent 1. Linearity, accuracy and precision for the analysis were confirmed. Extracts of dose formulations above 1.0 μg/g in diet were diluted to the target concentration of 0.2 μg/mL with diluent 1.

The Lower Limit of Quantitation (LLOQ) of the method was 0.1 μg/mL of Butylparaben in diluent 1, the lowest calibration standard. The Limit of Detection (LOD) was estimated to be approximately 0.0051 μg/mL Butylparaben in diluent 1, calculated before any corrections for dilution factors. Analysis of replicate blank vehicle samples indicated no interference peaks.

Overall, results for this validation indicate that the assay procedures were sufficiently linear, reproducible and accurate to support dose formulation analyses by GC/MS.

Pre-study samples for this study were analyzed for concentration and homogeneity verification according to the method described in Charles River Laboratories Discovery and Development Services Worcester Division Laboratory Method (LM) for the "Analysis of Butylparaben in CE-2 Diet Dose Formulations by GC/MS" LM BUTY00. A copy of the most recent LM is included in Appendix B. Results indicated that the formulations were prepared accurately.

After the initial sample analysis, the sensitivity for the GC/MS decreased. As a result, new procedures were developed and validated for the analysis of Butylparaben in CE-2 Diet formulations by High Performance Liquid Chromatography (LC) with MS detection.

The procedures are applicable for the analysis of dose formulations concentration range of 92 μg/g to 14900 μg/g of butylparaben in CE-2 diet. Validation of the butylparaben formulation analysis was performed using a range of calibration standards spanning approximately 0.8 to 7.0 μg/mL of butylparaben in diluent 1. Linearity, accuracy and precision for the analysis were confirmed. Extracts of dose formulations above 595 μg/g

in diet were diluted into the calibration range of the standard curve with 5% CE-2 diet extracts in diluent.

The Lower Limit of Quantitation (LLOQ) of the method was 0.8 µg/mL of butylparaben in diluent 1, the lowest calibration standard. The Limit of Detection (LOD) was estimated to be approximately 0.051 µg/mL butylparaben in diluent 1, calculated before any corrections for dilution factors. Analysis of replicate blank vehicle samples indicated no interference peaks.

Overall, results for this validation indicate that the assay procedures were sufficiently linear, reproducible and accurate to support dose formulation analyses by LC/MS.

Samples for this study were analyzed for concentration and stability verification according to the method described in Charles River Laboratories Discovery and Development Services Worcester Division Laboratory Method (LM) for the "Analysis of Butylparaben in CE-2 Rodent Diet Dose Formulations by HPLC/MS/MS" LM BUTY01. A copy of the most recent LM is included in Appendix B. Results indicated that the formulations were prepared accurately and are stable for at least 8 weeks at room temperature.

8. INTRODUCTION

The objective of this project was to develop and validate analytical procedures for the determination of levels of Butylparaben in CE-2 Diet. The procedures were used to analyze formulation samples from Charles River Laboratories Argus Division study 1203-006 (Charles River Laboratories Worcester Division Project Number PACA-ED00).

8.1. Experimental Design

The procedures described here involve analysis of Butylparaben by GC with MS detection (Part A) and analysis of Butylparaben by HPLC with MS detection (Part B). Calibration standards were prepared at known concentrations and analyzed to determine the accuracy, precision, specificity, linearity and limits of quantitation and detection for the methods. Formulations received from Charles River Laboratories Argus Division were analyzed for concentration, homogeneity and stability verification**.**

PART A: ANALYSIS OF BUTYLPARABEN BY GC/MS

9. MATERIALS AND METHODS

9.1. Computer Software

The GC data were acquired utilizing Hewlett-Packard ChemStation G1701BA software Version B.01.00. ChemStation software was used to integrate the peak areas of analyte and internal standard. Following integration the data was exported to a verified Excel 97 spreadsheet. The Excel 97 spreadsheet was used to perform the regression, calculate the regression constants and calculate the concentration of the analyte in unknown samples using the peak area ratios of analyte/internal standard. The tailing factor was determined by ChemStation software. The remaining system suitability parameters were calculated using Excel 97 spreadsheet.

9.2. Instrumentation

GC:	Hewlett Packard HP6890
Autosampler:	Hewlett Packard HP7683
Column:	Restek RTX-1, 15 m x 0.32 mm, 1 μm film thickness
Detector:	Hewlett Packard HP 5973 MSD

9.3. Preparation of Reagents and Standards

Refer to the Laboratory Method in Appendix B for the preparation of reagents and standards. During the method validation, the calibration standards were prepared at three concentration levels: 50%, 100% and 150% of target concentration. Each calibration standard and blank was prepared in triplicate.

9.4. Analytical Formulations for the 1:10 and 1:10,000 Dilutions

One stock solution of Butylparaben was prepared at approximately 130 µg/mL by weighing approximately 13000 µg of Butylparaben into a 100 mL volumetric flask and diluting to volume with diluent 1. A working stock solution was prepared at a concentration of approximately 2.6 µg/mL by pipetting 1.0 mL of the stock into a 50 mL volumetric flask and diluting to volume with diluent 1. Replicate low level dilution verification extracts were prepared by pipetting 0.4 mL aliquots of the working stock into four individual 50 mL polyethylene tubes which contained approximately 2 g of blank CE-2 diet and adding 9.6 mL of diluent 1. Refer to the Laboratory Method in Appendix B for the extraction procedure. Aliquots of the resulting supernatant were transferred into individual autosampler vials for analysis.

High level dilution verification extracts were prepared at approximately 10,000 µg/g by weighing approximately 20,000 µg of Butylparaben into four individual 50 mL polyethylene tubes which contained approximately 2 g of blank CE-2 diet. An aliquot of diluent 1 (10 mL) was added to each tube. Refer to the Laboratory Method in Appendix B for the extraction procedure. A dilution of each extract was prepared by pipetting an aliquot (1.0 mL) of each supernatant into individual 100 mL volumetric flasks. The flasks were brought to volume with diluent 1. A final dilution was prepared by pipetting a 1 mL aliquot of the initial dilution into a 100 mL volumetric flask. The flask was brought to volume with diluent 1. Aliquots of each final dilution were transferred into individual autosampler vials for analysis.

Resultant concentrations for the low and high dilution verification solutions were approximately 0.1 µg/mL and 0.2 µg/mL of Butylparaben in extract solution, respectively.

9.5. Calibration Standards

Refer to the laboratory method in Appendix B for the procedures concerning preparation of standards.

9.6. Preparation of Solution Standards

One set of solution standards was prepared for recovery evaluation using the same stocks as the calibration standards. The solution standards were prepared the same as the calibration standards except the final solutions did not contain any vehicle and no extraction procedure was performed.

9.7. Preparation of Dose Formulation Samples

Samples from Charles River Laboratories Argus Division study 1203-006 were received on June 16, June 29 and July 7, 2004. The samples were received in individual vials,

each containing approximately 25 g, and all were in good condition. All samples were received at ambient temperature. Samples which were not analyzed immediately were stored at room temperature (22±5°C) until analysis. Refer to the Laboratory Method in Appendix B for the procedures concerning preparation of samples for analysis. Samples received on June 16, 2004 were analyzed by GC/MS. Samples received on June 29 and July 7, 2004 were analyzed by LC/MS.

Refer to the Laboratory Method in Appendix B for chromatographic conditions and calculations.

10. RESULTS AND DISCUSSION

10.1. Method Development

The methods were developed using general methodology provided by the Sponsor.

10.2. Validation Results

Refer to Table 1 for tabulated results.

10.2.1. Recovery

Recovery values were calculated by comparison of the calibration standard peak area ratios (extracted with diet) to peak area ratios obtained for solution standards. The Butylparaben mean recovery for the concentration range was 103.1% (RSD = 2.5%). Individual Butylparaben values ranged from 101.0% to 105.9%.

10.2.2. Linearity

The assay was linear within the range tested of approximately 0.10 to 0.30 µg/mL of Butylparaben in diluent. Refer to the plot in Figure 1, which shows the unweighted linear regression graph with the actual calibration standard data points for the validation analysis run. Linearity was also demonstrated by the correlation coefficient obtained, which was greater than 0.998, and the lack of bias in the calculated percent error values for the calibration standards. These percent errors ranged from –4.4% to 4.8%**.**

10.2.3. Accuracy

Accuracy of the method was evaluated by the analysis of four replicates of the low and high concentration dilution verification solutions**.** Mean concentrations found for the low concentration dilution verification solutions during the run were compared to theoretical concentrations and expressed as percent errors. A value of –2.9% was obtained. Mean percent error for the high concentration dilution verification solutions was calculated by determining the bias of the mean found/theoretical concentrations. A value of –2.7% was obtained. Accuracy of the method was also evaluated by the back-calculated results for the calibration standards using the linear regression standard curve. Concentrations were compared to theoretical concentrations and expressed as percent errors. These percent

errors ranged from –4.4% to 4.8%. Mean accuracy values at the low and high end of the calibration range were 3.0% and 1.0%, respectively.

10.2.4. Precision

Within the run, precision was evaluated by the analysis of four replicates of the low and high concentration dilution verification solutions**.** The relative standard deviations (RSD) of the replicates were calculated. Values of 2.4% and 1.0% RSD were obtained for the low and high dilution verification solutions, respectively.

10.2.5. Sensitivity

The Lower Limit of Quantitation (LLOQ) for the analysis was defined as 0.10 µg/mL of Butylparaben in diluent 1, the lowest calibration standard. The RSD obtained for triplicate calibration standards at this level was determined to be 1.8%. The LOD for undiluted samples was estimated to be 0.051 µg/mL, calculated as three times the standard deviation of the back-calculated concentration of the low calibration standard.

10.2.6. Specificity

Specificity was demonstrated by the lack of any significant interfering chromatographic peaks found in three blank CE-2 diet extract samples. Refer to Figure 3 for an example chromatogram.

10.2.7. Summary

Overall, results for the validation indicated that the procedure was sufficiently linear, reproducible, accurate and specific to support analyses of dose formulation samples.

10.3. Concentration and Homogeneity Results

Refer to the Dose Formulation Analysis Report in Appendix A for details. The report consists of results and conclusions from one analysis period. Preparation and analysis dates are listed for each result along with Charles River Laboratories Argus Division sample identification.

10.3.1. Concentration

Test article samples prepared on June 15, 2004, which were used for dosing, were within acceptable limits of ±15% error.

10.3.2. Homogeneity

Homogeneity was determined for all dose formulation concentration levels. Mean concentration results from samples taken from the top, middle and bottom of the formulations were calculated. Homogeneity RSD was calculated by determining the percent relative standard deviation of the three mean values. All of the results were within the acceptable range of ≤5% RSD. The values obtained were 1.8%, 0.8% and 5.0% RSD for the 100, 1000 and 10,000 ppm formulations, respectively.

PART B: ANALYSIS OF BUTYLPARABEN BY LC/MS

11. MATERIALS AND METHODS

11.1. Computer Software

The LC/MS data were acquired utilizing Applied Biosystems/MDS SCIEX Analyst software version 1.3.1. Analyst software was used to integrate the peak areas of analyte and internal standard and calculate the peak area ratios. Following integration the data was exported to a verified Excel 97 spreadsheet. The Excel 97 spreadsheet was used to perform the regression, calculate the regression constants and calculate the concentration of the analyte in unknown samples using the peak area ratios of the analyte relative to the internal standard. The tailing factor was manually calculated and the remaining system suitability parameters were determined utilizing Excel 97 spreadsheet.

11.2. Instrumentation

Pump:	Agilent 1100 LC Binary Pump, Model G1312A
Autosampler:	HTS PAL CTC Analytics, Leap Technologies
Column:	Phenomenex Hypersil BDS C18, 50 x 4.6 mm, 3 µ
Pre-Column Frit:	Upchurch Scientific, SS frit, 0.094 x 0.25 mm
Mass Spectrometer:	PE/Sciex API 300
Interface:	Atmospheric Pressure Chemical Ionization, Positive-Ion Mode
Scan Mode:	Multiple Reaction Monitoring (MRM)

11.3. Preparation of Reagents and Standards

Refer to the Laboratory Method in Appendix B for the preparation of reagents and standards. During the method validation the lowest and highest calibration standards were prepared in quadruplicate and the blank was prepared in triplicate.

11.4. Analytical Formulations for the 1:20 and 1:500 Dilutions

One stock solution of Butylparaben was prepared at approximately 400 µg/mL by weighing approximately 20,000 µg of Butylparaben into a 50 mL volumetric flask and diluting to volume with diluent 1. Replicate low level dilution verification extracts were prepared by pipetting 0.5 mL aliquots of the stock into four individual 50 mL polyethylene tubes which contained 2 g of blank CE-2 diet. An aliquot (9.5 mL) of diluent 1 was added to each tube. Refer to the Laboratory Method in Appendix B for the extraction procedure. After extraction, a 1 mL aliquot of supernatant was pipetted into individual 20 mL volumetric flasks and brought to volume with 5% CE-2 diet extract in diluent. Aliquots of each final dilution were transferred into individual autosampler vials for analysis.

High level dilution verification extracts were prepared at approximately 10,000 µg/g by weighing approximately 20,000 µg of Butylparaben into four individual 50 mL polyethylene tubes containing approximately 2 g of blank CE-2 diet. An aliquot (10 mL) of diluent 1 was added to each tube. Refer to the Laboratory Method in Appendix B for the extraction procedure. A dilution of each extract was prepared by pipetting 1.0 mL aliquots of supernatant into individual 20 mL volumetric flasks. The flasks were brought to volume with diluent 1. A final dilution was prepared by pipetting a 1 mL aliquot of the dilution into individual 25 mL volumetric flasks and bringing to volume with 5% CE-2 diet extract in diluent. Aliquots of each final dilution were transferred into individual autosampler vials for analysis.

Resultant concentrations for the low and high dilution verification solutions were approximately 1.0 µg/mL and 4.0 µg/mL of Butylparaben in extract solution, respectively.

11.5. Calibration Standards

Refer to the laboratory method in Appendix B for the procedures concerning preparation of standards.

11.6. Preparation of Solution Standards

One set of six solution standards was prepared for recovery evaluation using the same stocks as the calibration standards. The solution standards were prepared the same as the calibration standards except the final solutions did not contain any vehicle and no extraction was performed.

11.7. Preparation of Post-Extraction Standards

The post-extraction standards were prepared the same as the solution standards except 5% CE-2 diet extract in diluent was used to bring the flasks to volume. The amount of diet extract contained in the post-extraction standards was proportional to that contained in the extracted calibration standards and samples.

11.8. Preparation of Dose Formulation Samples

Refer to the Laboratory Method in Appendix B for the procedures concerning preparation of samples for analysis. Samples received on June 29 and July 7, 2004 were analyzed by LC/MS.

Refer to the Laboratory Method in Appendix B for chromatographic conditions and calculations.

12. RESULTS AND DISCUSSION

12.1. Method Development

The methods were developed using general methodology provided by the Sponsor.

12.2. Validation Results

Refer to Table 2 for tabulated results.

12.2.1. Recovery

Combined recovery values were calculated by comparison of the calibration standard peak areas (extracted with diet) to peak areas obtained for solution standards. True recovery values were calculated by comparison of the calibration standard peak areas (extracted with diet) to peak areas obtained for post-extraction standards (not extracted but containing 5% CE-2 diet extract in diluent). Instrument suppression/enhancement values were calculated by comparison of solution standards to peak areas obtained for post-extraction standards.

The Butylparaben mean combined recovery, true recovery and suppression/enhancement values for the six standard concentrations were 107.6%, 99.2% and 95.8%, respectively.

The Isopropyl Paraben (internal standard) mean combined recovery, true recovery and suppression/enhancement values for the six standard concentrations were 110.3%, 98.5% and 100.1%, respectively.

Recovery values were calculated by comparison of the calibration standard peak area ratios (extracted with diet) to peak area ratios obtained for solution standards. The Butylparaben mean recovery for the concentration range was 95.8% (RSD = 7.9%). Individual Butylparaben values ranged from 84.7% to 104.1%.

12.2.2. Linearity

The assay was linear within the range tested of approximately 0.80 to 7.0 µg/mL of Butylparaben in diluent. Refer to the plot in Figure 2, which shows the unweighted linear regression graph with the actual calibration standard data points for the validation analysis run. Linearity was also demonstrated by the correlation coefficient obtained, which was greater than 0.998, and the lack of bias in the calculated percent error values for the calibration standards. These percent errors ranged from –6.0% to 4.8%.

12.2.3. Accuracy

Accuracy of the method was evaluated by the analysis of four replicates of the low and high concentration dilution verification solutions. Mean concentrations found during the run were compared to theoretical concentrations and expressed as percent errors. Values of 2.0% for the low and 0.2% for the high concentration were obtained. Accuracy of the

method was also evaluated by the back-calculated results for the calibration standards using the linear regression standard curve. Concentrations were compared to theoretical concentrations and expressed as percent errors. These percent errors ranged from –6.0% to 4.8%. Mean accuracy values at the low and high end of the calibration range were -3.1% and –1.6%, respectively.

12.2.4. Precision

Within the run, precision was evaluated by the analysis of four replicates of the low and high concentration dilution verification solutions. The relative standard deviations (RSD) of the replicates were calculated. Values of 6.8% and 1.4% RSD were obtained for the low and high dilution verification solutions, respectively.

12.2.5. Sensitivity

The Lower Limit of Quantitation (LLOQ) for the analysis was defined as 0.80 μg/mL of Butylparaben in diluent 1, the lowest calibration standard. The RSD obtained for quadruplicate calibration standards at this level was determined to be 2.3%. The LOD for undiluted samples was estimated to be 0.051 μg/mL, calculated as three times the standard deviation of the back-calculated concentration of the low calibration standard.

12.2.6. Specificity

Specificity was demonstrated by the lack of any significant interfering chromatographic peaks found in three blank CE-2 diet extract samples. Refer to Figure 4 for an example chromatogram.

12.2.7. Summary

Overall, results for the validation indicated that the procedure was sufficiently linear, reproducible, accurate and specific to support analyses of dose formulation samples.

12.3. Concentration and Stability Results

Refer to the Dose Formulation Analysis Reports in Appendix A for details. Each report consists of results and conclusions from one analysis period. Preparation and analysis dates are listed for each result along with Charles River Laboratories Argus Division sample identification.

12.3.1. Concentration

Test article samples prepared on June 28 and July 5, 2004, which were used for dosing, were within acceptable limits of ±15% error.

12.3.2. Stability

The stability was evaluated for the low and high dose formulation concentration levels. Initial stability samples were analyzed on June 18, 2004 by GC/MS. Stability samples were stored at 22±5°C and analyzed on July 13, and August 13, 2004 by LC/MS

resulting in 25 day and 8 week stability periods. The final stability sample results were compared with the initial results and expressed as percent errors. All of the results were within the acceptable range of ±10% error.

TABLES

Table 1. GC/MS Validation Results

Analyzed on June 16, 2004

Concentration in μg/mL

Calibration Standard Results:

Standard Description	Theoretical Conc.	Response Area Ratio	Conc. Found	% Error
Blank A	0	0	ND	-
Blank B	0	0	ND	-
Blank C	0	0	ND	-
Cal Std A1a	0.1037	0.06747	0.1054	+1.6%
Cal Std A1b	0.1037	0.06986	0.1087	+4.8%
Cal Std A1c	0.1037	0.06820	0.1064	+2.6%
Cal Std A2a	0.2074	0.14003	0.2048	-1.3%
Cal Std A2b	0.2074	0.13515	0.1982	-4.4%
Cal Std A2c	0.2074	0.13689	0.2005	-3.3%
Cal Std A3a	0.3110	0.22229	0.3176	+2.1%
Cal Std A3b	0.3110	0.21805	0.3118	+0.3%
Cal Std A3c	0.3110	0.21886	0.3129	+0.6%

Slope:	0.72948
Y-Int:	-0.0094031
Corr:	0.99829
n:	9

Std	Response RSD	Mean % Error
A1	1.8%	3.0%
A2	1.8%	-3.0%
A3	1.0%	1.0%

ND = None detected

Table 1. GC/MS Validation Results (Concluded)

Analyzed on June 16, 2004

Concentration in µg/mL

Solution Standard Recovery Results:

Standard Description	Theoretical Conc.	Spiked Calibration Standards Avg. Area Ratio	Solution Standards Area Ratio	% Recovery
Cal Std A1a	0.1037	0.068510	0.06467	105.9%
Cal Std A2a	0.2074	0.13736	0.13600	101.0%
Cal Std A3a	0.3110	0.21973	0.21490	102.2%
			Average Recovery:	103.1%
			RSD:	2.5%

Concentrations in µg/g

Dilution Verification Results:

Theoretical Conc.	Replicate	Response Area Ratio	Found Conc.
0.5184	A	0.06647	0.5201
	B	0.06436	0.5056
	C	0.06310	0.4970
	D	0.06237	0.4920
		Mean:	0.5037
		RSD:	2.4%
		% Error:	-2.9%

Theoretical Conc.	Replicate	Response Area Ratio	Found Conc.	% Found./%Theor.
10025	A	0.13162	9666	96.4%
10100	B	0.13576	9950	98.5%
10010	C	0.13182	9680	96.7%
10355	D	0.13829	10120	97.7%
			Mean:	97.3%
			RSD:	1.0%
			% Error:	-2.7%

Table 2. LC/MS Validation Results

Analyzed on July 12, 2004

Concentration in μg/mL

Calibration Standard Results:

Standard Description	Theoretical Conc.	Response Area Ratio	Conc. Found	% Error
Blank A	0	0	ND	-
Blank B	0	0	ND	-
Blank C	0	0	ND	-
EX Std A1a	0.7988	0.16372	0.7707	-3.5%
EX Std A1b	0.7988	0.16971	0.7974	-0.2%
EX Std A1c	0.7988	0.16417	0.7727	-3.3%
EX Std A1d	0.7988	0.16061	0.7569	-5.2%
EX Std B1	2.001	0.41319	1.881	-6.0%
EX Std A2	3.195	0.70287	3.171	-0.8%
EX Std B2	4.002	0.85718	3.858	-3.6%
EX Std A3	5.592	1.30687	5.860	+4.8%
EX Std B3a	7.003	1.53113	6.858	-2.1%
EX Std B3b	7.003	1.53542	6.877	-1.8%
EX Std B3c	7.003	1.54968	6.941	-0.9%
EX Std B3d	7.003	1.53701	6.885	-1.7%
EX Std A1a	0.7988	0.17791	0.8339	+4.4%
EX Std B1	2.001	0.44456	2.021	+1.0%
EX Std A2	3.195	0.72514	3.270	+2.3%
EX Std B2	4.002	0.91948	4.135	+3.3%
EX Std A3	5.592	1.30138	5.836	+4.4%
EX Std B3a	7.003	1.57702	7.063	+0.9%

Slope:	0.22462
Y-Int:	-0.0093954
Corr:	0.99885
n:	18

Std	Area Ratio RSD	Mean % Error
A1	2.3%	-3.1%
B3	0.5%	-1.6%

ND = None detected

Table 2. LC/MS Validation Results (Continued)

Analyzed on July 12, 2004

Concentration in μg/mL

Butylparaben Recovery Results:

Standard Description	Theoretical Conc.	Extracted Standards (Peak Area)	Solution Standards (Peak Area)	Post-Extraction Standards (Peak Area)	Combined Recovery[a]	Suppression/ Enhancement[b]	True Recovery[c]
A1	0.7988	6129	6004	7131	102.1%	118.8%	85.9%
B1	2.001	18512	15308	17659	120.9%	86.7%	104.8%
A2	3.195	30234	28801	26598	105.0%	92.4%	113.7%
B2	4.002	33249	28808	34091	115.4%	84.5%	97.5%
A3	5.592	48957	46160	52343	106.1%	88.2%	93.5%
B3	7.003	60507	62940	60487	96.1%	104.1%	100.0%
			Mean Recovery:		107.6%	95.8%	99.2%

Isopropyl Paraben (Internal Standard) Recovery Results:

Standard Description	Theoretical Conc.	Extracted Standards (Peak Area)	Solution Standards (Peak Area)	Post-Extraction Standards (Peak Area)	Combined Recovery[a]	Suppression/ Enhancement[b]	True Recovery[c]
A1	3.032	36532	30919	39357	118.2%	127.3%	92.8%
B1	3.032	43003	36080	40651	119.2%	88.8%	105.8%
A2	3.032	42256	36635	39419	115.3%	107.6%	107.2%
B2	3.032	37250	34681	37420	107.4%	92.7%	99.5%
A3	3.032	37550	36770	42303	102.1%	86.9%	88.8%
B3	3.032	39097	39311	40452	99.5%	97.2%	96.6%
			Mean Recovery:		110.3%	100.1%	98.5%

[a] = Combined recovery calculated by comparison of the mean peak areas for extracted standards to the mean peak areas for solution standards.

[b] = Suppression/enhancement calculated by comparison of the mean peak areas for post-extraction standards to the mean peak areas for solution standards. Note: A value greater than 100% indicates signal enhancement whereas a value below 100% indicates signal suppression.

[c] = True recovery calculated by comparison of the mean peak areas for extracted standards to the mean peak areas for post-extraction standards.

Table 2. LC/MS Validation Results (Concluded)

Analyzed on July 12, 2004

Concentration in µg/mL

Solution Standard Recovery Results:

Standard Description	Theoretical Conc.	Spiked Calibration Standards Avg. Area Ratio	Solution Standards Area Ratio	% Recovery
Cal Std A1	0.7988	0.16455	0.19419	84.7%
Cal Std B1	2.0008	0.41319	0.42426	97.4%
Cal Std A2	3.1952	0.70287	0.78616	89.4%
Cal Std B2	4.0016	0.85718	0.83067	103.2%
Cal Std A3	5.5916	1.30687	1.25538	104.1%
Cal Std B3	7.0028	1.53831	1.60107	96.1%
			Average Recovery:	95.8%
			RSD:	7.9%

Concentrations in µg/g

Dilution Verification Results:

Theoretical Conc.	Replicate	Response Area Ratio	Found Conc.
99.85	A	0.23830	110.3
	B	0.20056	93.47
	C	0.21663	100.6
	D	0.22177	102.9
		Mean:	101.8
		RSD:	6.8%
		% Error:	+2.0%

Theoretical Conc.	Replicate	Response Area Ratio	Found Conc.	% Found/Theor.
9985	A	0.90168	10140	101.6%
10150	B	0.91232	10260	101.1%
10750	C	0.94000	10570	98.3%
9875	D	0.87726	9869	99.9%
			Mean:	100.2%
			RSD:	1.4%
			% Error:	+0.2%

FIGURES

Figure 1. Standard Curve for Validation Run for GC/MS

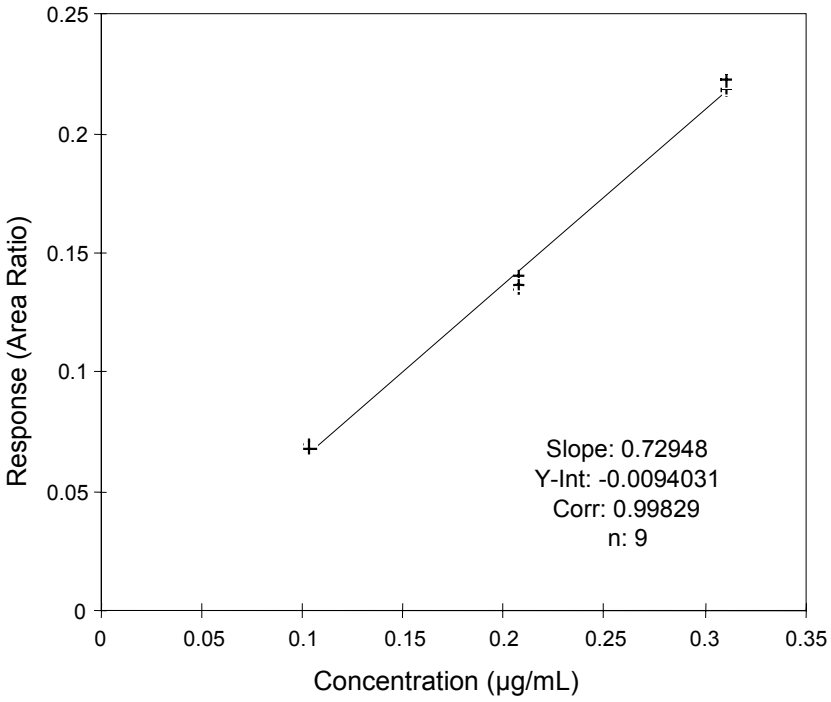


Figure 2. Standard Curve for Validation Run for LC/MS

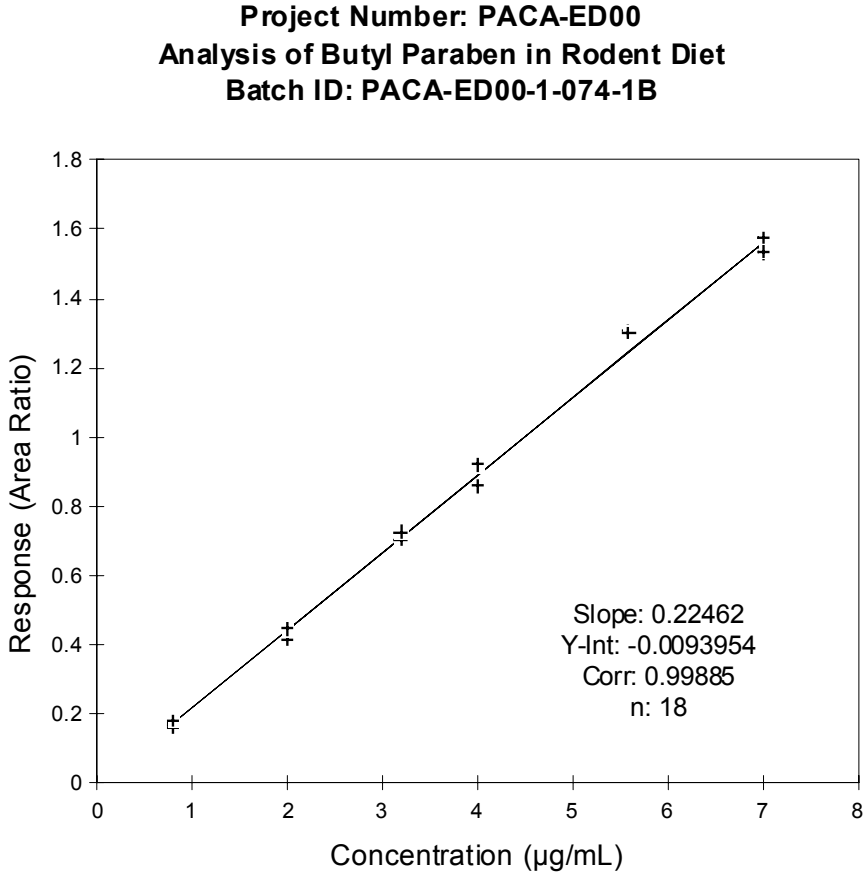
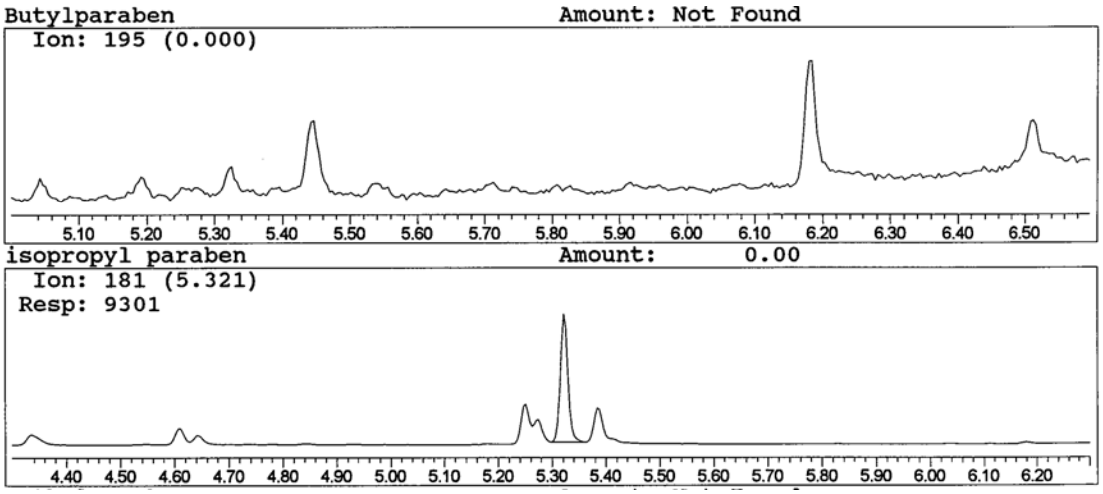


Figure 3. Example Chromatogram for GC/MS

Study #: 1203-006
Run ID: Validation
Inj. Vol.: 2 µL
Column Manuf: Restek
Column Length: 15 m
Film Thickness: 1 µm
Ion Source: CI
MS Source Temp: 250°C
Auxillary: On @ 280°C
Analysis Date: June 16, 2004
Notebook Ref.: PACA-ED00-1-046-1
Column #: 524772B
Type: RTX-1
Column ID: 0.32 mm
Carrier Gas: Helium @ 4.0 mL/min
CI Gas: Methane
MS Quad Temp: 106°C
Oven Program: 70°C for 1.50 min, ramp 30°C/min to 300°C, 1 minute hold

Sample ID: Blank File 10461030.D (Sensitivity: ~70,000 mv FS)



Sample ID: Standard B3 File 10461018.D (Sensitivity: ~90,000 mv FS)

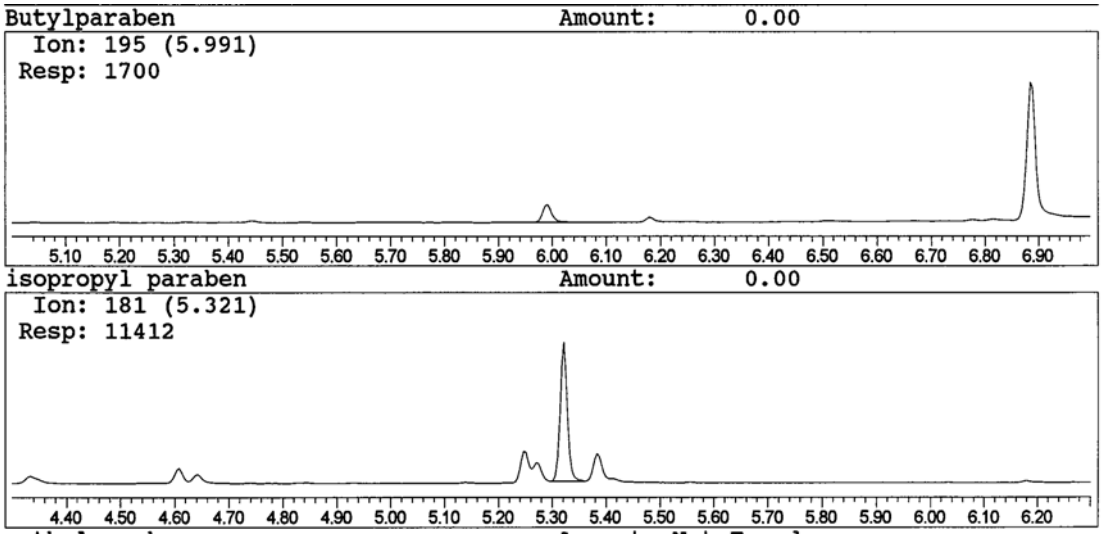
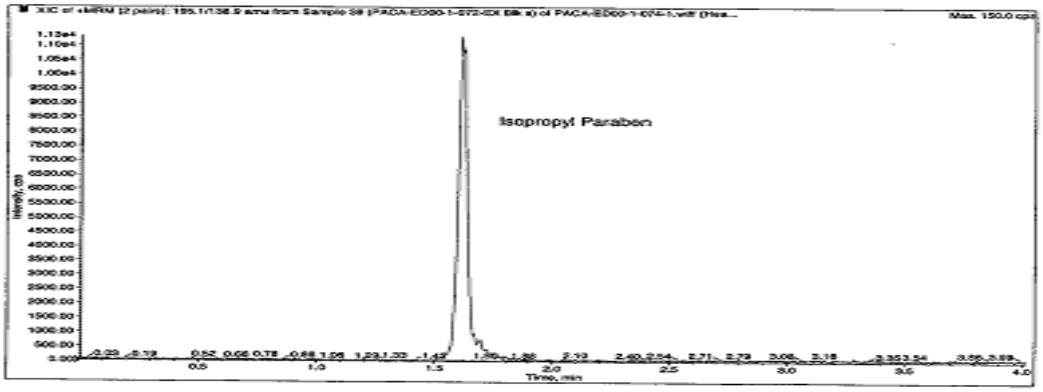


Figure 4. Example Chromatogram for LC/MS

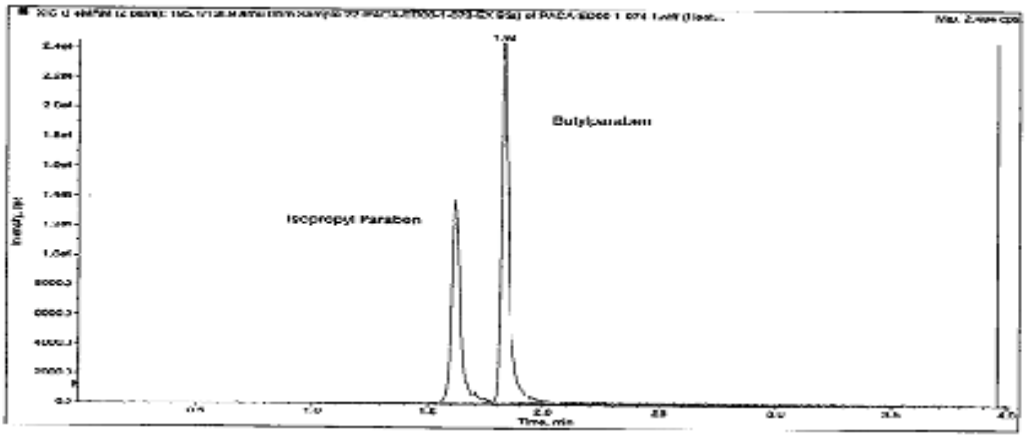
Study #: 1203-006
Run ID: Validation
Inj. Vol.: 15 µL
Column Manuf: Phenomenex
Column Length: 50 mm
Flow Rate: 1.0 mL/min
Detection: MS
Interface: APCI, Positive Ion Mode
Analysis Date: July 12, 2004
Notebook Ref.: PACA-ED00-1-074-1
Column #: 240423-7
Packing: Hypersil BDS C18
Column ID: 4.6 mm
Particle Size: 3 micron
Pressure: ~950 psi
Scan Mode: MRM
Pre-Column Frit: Upchurch Scientific, SS frit, 0.094 x 0.25 mm

Mobile Phase A: 0.1% Formic Acid in Water
Mobile Phase B: 0.1% Formic Acid in Acetonitrile

Sample ID: Blank (Sensitivity: 11,300 cps FS)



Sample ID: Standard B3 (Sensitivity: 24,000 cps FS)



Appendix A. Dose Formulation Analysis Reports

DOSE FORMULATION ANALYSIS REPORT

Sponsor: CTFA
Study Facility: Argus Division
Protocol Number: 1203-006
Analyte: Butylparaben
Analytical Facility: Worcester Division
Batch ID: PACA-ED00-1-051-1
Sampling Criteria: Prestudy Concentration and Homogeneity Analysis
Vehicle: CE-2 Diet
Storage Conditions: 22±5°C
Laboratory Method: LM # BUTY00, Revision 00 (Draft)
Analysis Date: June 18, 2004

RESULTS: (Standard concentrations in µg/mL, sample concentrations in µg/g)

CALIBRATION STANDARDS

Standard Description	Nominal Conc.	Response Area Ratio	Calculated Conc.	% Bias	Criteria Limit	Standard Pass/Fail
Cal Std	0.2101	0.13034	0.2058	-2.0%	10%	PASS
Cal Std	0.2101	0.13452	0.2124	+1.1%	10%	PASS
Cal Std	0.2101	0.13665	0.2157	+2.7%	10%	PASS
Cal Std	0.2101	0.12745	0.2012	-4.2%	10%	PASS
Cal Std	0.2101	0.13645	0.2154	+2.5%	10%	PASS

Mean Response Factor = 0.6334
Standard Agreement = 0.3%

CHECK STANDARDS

Standard Description	Nominal Conc.	Response Area Ratio	Calculated Conc.	% Bias	Criteria Limit	Standard Pass/Fail
Check Std	0.2101	0.13776	0.2175	3.5%	5.0%	PASS
Check Std	0.2101	0.13636	0.2153	2.5%	5.0%	PASS
Check Std	0.2101	0.12929	0.2041	-2.9%	5.0%	PASS

SAMPLES

Sample Description	Prep Date	Nominal Sample Conc.	Replicate	Response Area Ratio	Dilution Factor	Density Corrected µg/g	Mean µg/g Found	% Bias
Group I Top	06/15/04	0	A	0	1	ND		
			B	0	1	ND		
Group I Mid	06/15/04	0	A	0	1	ND		
			B	0	1	ND		
Group I Bot	06/15/04	0	A	0	1	ND		
			B	0	1	ND		
Group II Top	06/15/04	100	A	0.1400	100	109.9	107.5	+7.5%
			B	0.1358	100	105.1		
Group II Mid	06/15/04	100	A	0.1312	100	102.0	105.4	+5.4%
			B	0.1359	100	108.8		
Group II Bot	06/15/04	100	A	0.1355	100	105.1	103.8	+3.8%
			B	0.1289	100	102.4		
Group III Top	06/15/04	1000	A	0.1357	1000	1048	1037	+3.7%
			B	0.1286	1000	1026		
Group III Mid	06/15/04	1000	A	0.1229	1000	980.8	1030	+3.0%
			B	0.1354	1000	1080		
Group III Bot	06/15/04	1000	A	0.1357	1000	1078	1047	+4.7%
			B	0.1299	1000	1016		
Group IV Top	06/15/04	10000	A	0.1368	10000	10860	10630	+6.3%
			B	0.1307	10000	10400		
Group IV Mid	06/15/04	10000	A	0.1240	10000	9929	9672	-3.3%
			B	0.1219	10000	9414		
Group IV Bot	06/15/04	10000	A	0.1271	10000	9925	10450	+4.5%
			B	0.1375	10000	10970		

Homogeneity

Sample Description	Nominal Sample Conc.	Grand Mean Conc.	RSD	% Error
Group II	100	105.6	1.8%	5.6%
Group III	1000	1038	0.8%	3.8%
Group IV	10000	10250	5.0%	2.5%

CONCLUSIONS: Results indicate that the concentrations of the dose formulations are within the acceptable limits of ±15% of theoretical concentrations. The formulations are also within the acceptable limits of ≤5% RSD for homogeneity.

ACTIONS TAKEN: None.

DOSE FORMULATION ANALYSIS REPORT

Sponsor: CTFA
Study Facility: Argus Division
Protocol Number: 1203-006
Analyte: Butylparaben
Analytical Facility: Worcester Division
Batch ID: PACA-ED00-1-080-1
Sampling Criteria: Concentration Analysis and 25-Days Stability Analysis
Vehicle: CE-2 Diet
Storage Conditions: 22±5°C
Laboratory Method: LM #BUTY01 Revision 00 (draft)
Analysis Date: July 13, 2004
Note: Nominal sample concentrations were obtained from mean found concentrations obtained in batch PACA-ED00-1-051-1 analyzed on June 18, 2004.

RESULTS: (Standard concentrations in µg/mL, sample concentrations in µg/g, ND = none detected)

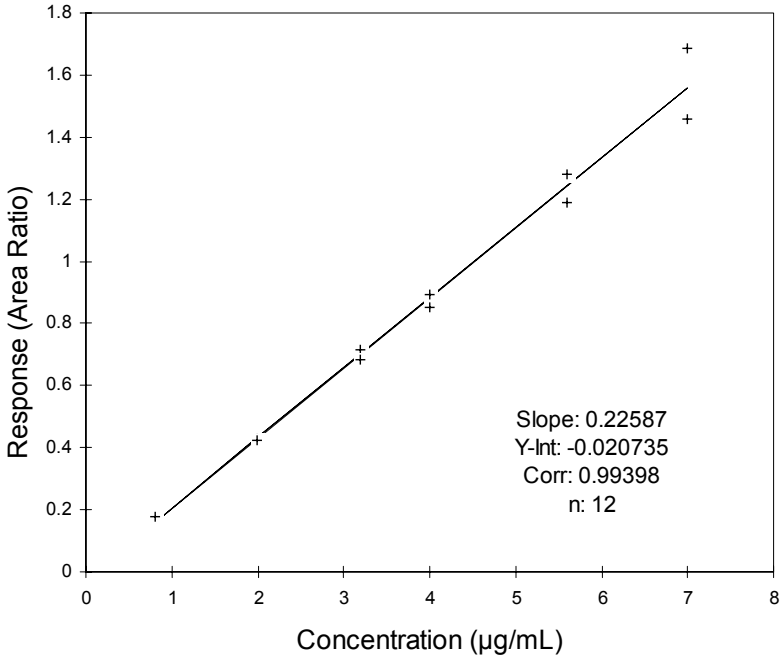
CALIBRATION STANDARDS

Standard Description	Nominal Conc.	Response Area Ratio	Calculated Conc.	% Bias	"X" = Exclude	Criteria Limit	Standard Pass/Fail
EX Std A1	0.7988	0.16810	0.8360	+4.7%		10%	PASS
EX Std B1	1.999	0.42875	1.990	-0.5%		10%	PASS
EX Std A2	3.195	0.71474	3.256	+1.9%		10%	PASS
EX Std B2	3.998	0.89449	4.052	+1.4%		10%	PASS
EX Std A3	5.592	1.18971	5.359	-4.2%		10%	PASS
EX Std B3	6.997	1.45908	6.552	-6.4%		10%	PASS
EX Std A1	0.7988	0.17588	0.8705	+9.0%		10%	PASS
EX Std B1	1.999	0.42190	1.960	-2.0%		10%	PASS
EX Std A2	3.195	0.68218	3.112	-2.6%		10%	PASS
EX Std B2	3.998	0.85047	3.857	-3.5%		10%	PASS
EX Std A3	5.592	1.28036	5.760	+3.0%		10%	PASS
EX Std B3	6.997	1.68567	7.555	+8.0%		10%	PASS

CHECK STANDARDS

Standard Description	Nominal Conc.	Response Area Ratio	Dilution Factor	Conc. Found	% Bias	Criteria Limit	Standard Pass/Fail
A3 Check Std	5.592	1.23245	1	5.548	-0.8%	10.0%	PASS
A3 Check Std	5.592	1.33357	1	5.996	+7.2%	10.0%	PASS

Project Number: PACA-ED00
Analysis of Butyl Paraben in Rodent Diet
Batch ID: PACA-ED00-1-080-1



SAMPLES

Sample Description	Prep Date	Nominal Sample Conc.	Replicate	Response Area Ratio	Total Dilution Factor	Density Corrected µg/g	Mean µg/g Found	% Bias
Group I	06/28/04	0	A	0	100.9	ND		
			B	0	98.38	ND		
Group II	06/28/04	100	A	0.20006	100.0	97.76	98.98	-1.0%
			B	0.20351	100.9	100.2		
Group III	06/28/04	1000	A	0.45842	506.7	1075	1024	+2.4%
			B	0.41330	506.7	973.6		
Group IV	06/28/04	10000	A	0.88699	2514	10100	9910	-0.9%
			B	0.84096	2548	9719		
Group II (Stab)	06/15/04	105.6	A	0.22373	98.21	106.3	102.5	-2.9%
			B	0.20399	99.10	98.60		
Group IV (Stab)	06/15/04	10250	A	0.91070	2550	10520	10510	+2.5%
			B	0.91752	2526	10490		
Group I	07/05/04	0	A	0	99.74	ND		
			B	0	100.2	ND		
Group II	07/05/04	100	A	0.22941	99.73	110.4	108.1	+8.1%
			B	0.21658	100.6	105.7		
Group III	07/05/04	1000	A	0.45083	506.9	1058	1014	+1.4%
			B	0.41822	499.2	970.2		
Group IV	07/05/04	10000	A	0.88198	2482	9920	10030	+0.3%
			B	0.88104	2541	10140		

CONCLUSIONS: Results indicate that the formulations prepared on June 28 and July 5, 2004 are within the acceptable limits of ±15% of theoretical concentrations. The stability samples are within the acceptable limits of ±10% of initial found concentrations. The formulations are also stable for at least 25 days at room temperature.

ACTIONS TAKEN: None.

DOSE FORMULATION ANALYSIS REPORT

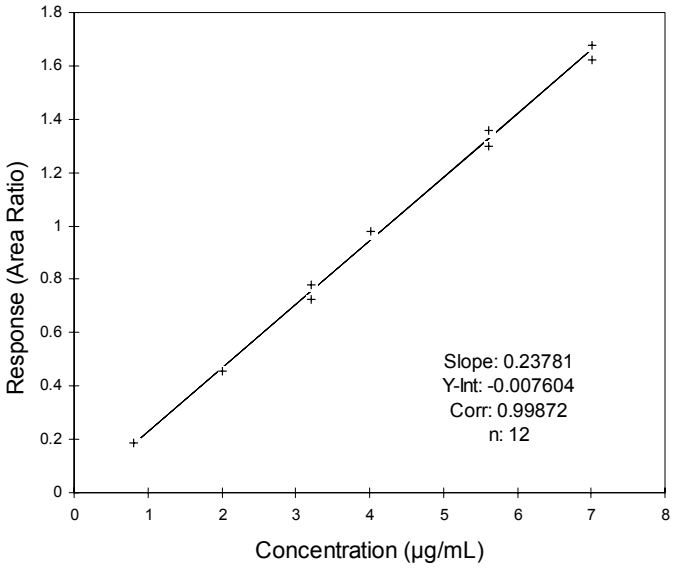
Sponsor: CTFA
Study Facility: Argus Division
Protocol Number: 1203-006
Analyte: Butylparaben
Analytical Facility: Worcester Division
Batch ID: PACA-ED00-2-006-1
Sampling Criteria: 8-Week Stability Analysis
Vehicle: CE-2 Diet
Storage Conditions: 22±5°C
Laboratory Method: LM #BUTY01 Revision 00
Analysis Date: August 13, 2004
Notes: Nominal sample concentrations were obtained from mean found concentrations obtained in batch PACA-ED00-1-051-1 analyzed on June 18, 2004.

RESULTS: (Standard concentrations in μg/mL, sample concentrations in μg/g)

CALIBRATION STANDARDS

Standard Description	Nominal Conc.	Response Area Ratio	Calculated Conc.	% Bias	"X" = Exclude	Criteria Limit	Standard Pass/Fail
Cal Std A1	0.8020	0.17760	0.7788	-2.9%		15%	PASS
Cal Std B1	2.006	0.45135	1.930	-3.8%		15%	PASS
Cal Std A2	3.208	0.7792	3.309	+3.1%		15%	PASS
Cal Std B2	4.011	0.97497	4.132	+3.0%		15%	PASS
Cal Std A3	5.614	1.2990	5.494	-2.1%		15%	PASS
Cal Std B3	7.020	1.6213	6.850	-2.4%		15%	PASS
Cal Std A1	0.8020	0.18677	0.8173	+1.9%		15%	PASS
Cal Std B1	2.006	0.45779	1.957	-2.4%		15%	PASS
Cal Std A2	3.208	0.72387	3.076	-4.1%		15%	PASS
Cal Std B2	4.011	0.97938	4.150	+3.5%		15%	PASS
Cal Std A3	5.614	1.3585	5.745	+2.3%		15%	PASS
Cal Std B3	7.020	1.6772	7.085	+0.9%		15%	PASS

Project Number: PACA-ED00
Analysis of Butylparaben in Rodent Diet
Batch ID: PACA-ED00-2-006-1



SAMPLES

Sample Description	Prep Date	Nominal Sample Conc.	Replicate	Response Area Ratio	Total Dilution Factor	Density Corrected µg/g	Mean µg/g Found	% Bias
Group II	06/15/04	105.6	A	0.22014	100.0	95.79	98.45	-6.8%
			B	0.23259	100.1	101.1		
Group IV	06/15/04	10250	A	0.90957	2492	9610	9596	-6.4%
			B	0.90225	2504	9581		

CONCLUSIONS: Results indicate that the formulations met acceptance criteria of ±10% of theoretical concentrations. The formulations are stable for at least 8 weeks at room temperature.

ACTIONS TAKEN: None.

Appendix B. Laboratory Methods

CHARLES RIVER
LABORATORIES
Discovery and Development Services
Worcester Division

LM Number:	BUTY00	Revision Number:	02		
Effective Date:	September 1, 2004	Page	1	Of	12

Laboratory Method for the Analysis of Butylparaben in CE-2 Diet Dose Formulations by GC/MS

Prepared By: Jennifer Bravo — 9/1/04
Jennifer Bravo, M.S. — **Date**
Senior Research Associate

Reviewed By: Dorothy Savage — 9/2/04
Dorothy Savage, B.S. — **Date**
Senior Scientist

Authorized By Release Note for S. Guyan — 9/2/04
Stephen A. Guyan, M.Sc. — **Date**
Senior Director, Analytical Chemistry Department

LM Number:	BUTY00	Revision Number:	02
Effective Date:	September 1, 2004	Page	2 Of 12

1 Purpose

The purpose of this laboratory method is to accurately determine the concentration of Butylparaben in CE-2 Diet dose formulations.

2 Scope

Analysis of Butylparaben in dose formulation samples with limitations as stated below.

Vehicle: CE-2 Diet
Sample Weight: 2 g
Volumetric Samples [ ] Gravimetric Samples [X] Both [ ]

Concentrations Covered by Laboratory Method:

Final Injected Concentration - µg/mL

LOD	0.0051
Target Concentration	0. 2 µg/mL
LLOQ to ULOQ	0. 1 – 0. 3

Corresponding Concentrations - µg/g (ppm) in CE-2 Diet

	Standard Extraction of 2 g	Additional 1 in 100 Dilution	Additional 1 in 1000 Dilution	Additional 1 in 10,000 Dilution
LOD	0. 051	5.1	51	510
LLOQ to ULOQ	1.0 – 3.0	100 – 300	1,000 – 3,100	10,000 – 31,000
Valid Sample Range	0, 1.0	100	1,000	10,000

3 Stability

Description	Concentration Range	Storage Conditions	Time Period
Process Stability	0.1 – 0.3 µg/mL	22 ± 5°C	24 hours
Stability Period 1	100 – 10,000 µg/g	22 ± 5°C	25 days
Stability Period 2	100 – 10,000 µg/g	22 ± 5°C	8 weeks

*Standards should be prepared fresh for each analysis until standard stability is established.

Note: All storage conditions are unprotected from light unless specified otherwise.

4 Definitions/Abbreviations

GC:	Gas Chromatography
IS:	Internal Standard (Isopropylparaben)
ND:	None detected
N/A:	Not applicable
SIM:	Selected Ion monitoring
MSD:	Mass Spectrometer Detector
LOD:	Limit of Detection
RPM:	Revolutions per minute
LLOQ:	Lower Limit of Quantitation
ULOQ:	Upper Limit of Quantitation
Purity/Salt Factor:	None – no correction
µg/g:	ppm
ACN:	Acetonitrile

5 Materials

5.1 Chemicals

Acetonitrile, HPLC grade or equivalent
CE-2 Diet, meal form from CLEA Japan, Inc.
Isopropylparaben, Sigma, CAS# 4191-7-3–5

5.2 Supplies

Volumetric flasks and pipets
50mL polypropylene conical test tubes
Autosampler Vial Caps
Teflon coated silica septa crimp caps

6 Procedure

6.1 Preparation of Reagents

Other volumes may be prepared using the same proportions. Store all reagents at room temperature and use within 14 days unless noted otherwise.

6.1.1 Needle Rinse

100% Acetonitrile

6.1.2 IS Stock Solution (0.4 mg/mL in ACN)

Weigh approximately 40 mg of isopropylparaben into a 100 mL volumetric flask. Bring to volume with ACN and mix until dissolved. Store at 5±3°C and discard after 90 days.

6.1.3 Diluent 1 (0.8 µg/mL IS in ACN)

A new batch of diluent should be prepared for each analysis. Transfer 4 mL of IS stock solution into a 2000 mL volumetric flask. Bring it to volume with ACN and mix well. Store at 5±3°C and discard after 7 days.

6.2 Preparation of Stocks, Working Stocks, Standards and Blanks

Stocks, working stocks, standards and blanks should be stored at 5 ± 3°C unless noted otherwise.

6.2.1 Preparation of Stocks

	Butylparaben weight (mg)*	Volumetric Flask (mL)	Diluent
Stock A	13	100	diluent 1
Stock B	13	100	diluent 1

* Record weights to the nearest 0.01 mg.

6.2.2 Preparation of Working Stocks

	Aliquot from Stock A (mL)	Aliquot from Stock B (mL)	Final Volume (mL)	Diluent
Working Stock A	1	N/A	50	diluent 1
Working Stock B	N/A	1	50	diluent 1

6.2.3 Preparation of Standard Spiking Solutions

	Aliquot from Working Stock A (mL)	Aliquot from Working Stock B (mL)	Final Volume (mL)	Diluent
Calibration Standard A1	2	N/A	25	diluent 1
Agreement Standard B1	N/A	2	25	diluent 1

6.2.4 Use the standard spiking solutions in section 6.2.3 to prepare matrix matched standards (section 6.3).

6.3 Standard Extract Preparation

6.3.1 Weigh approximately 2 g of blank CE-2 diet directly into tared 50 mL polypropylene conical test tubes using a balance capable of reading at least 0.01 g.

6.3.2 Using volumetric pipettes, add 10 mL of each standard spiking solution to individual tubes containing diet.

6.3.3 To prepare a matrix-matched blank, use a volumetric pipet to add 10 mL of diluent 1 to 2 g of feed.

6.3.4 Tightly cap the test tubes, vortex each for approximately 5 minutes, tumble in a rotary tumbler for approximately 30 minutes at a speed of approximately 30% and sonicate for approximately 15 minutes.

6.3.5 Centrifuge for 5 minutes at a speed of approximately 3000 rpm.

6.3.6 Remove the supernatent as soon as possible after the completion of the extraction and transfer to a 20 mL scintillation vial.

6.3.7 Transfer aliquots of each standard into individual crimp top autosampler vials.

6.4 Sample Extract Preparation

6.4.1 Prepare samples in duplicate by weighing approximately 2 g of CE-2 diet sample directly into tared 50 mL Polypropylene conical test tubes using a balance capable of reading at least 0.001 g.

6.4.2 Using volumetric pipettes, add 10 mL aliquots of diluent 1 to each test tube.

6.4.3 Tightly cap the test tubes, vortex each for approximately 5 minutes, tumble in a rotary tumbler for approximately 30 minutes at a speed of approximately 30% and sonicate for approximately 15 minutes.

6.4.4 Centrifuge for 5 minutes at a speed of approximately 3000 rpm.

6.4.5 Remove the supernatent as soon as possible after the completion of the extraction and transfer to a 20 mL scintillation vial.

6.4.6 The initial sample dilutions may be diluted further as indicated on the tables below. Mix well and transfer an aliquot of each final dilution into individual autosampler vials.

Second Dilution			
Sample Concentration Ranges	Aliquot from Initial Dilution (mL)	Final Dilution Volumetric Flask Size (mL)	Diluent
0 and 1.0 µg/g	N/A	N/A	N/A
100, 1,000 and 10,000 µg/g	1	100	diluent 1

Third Dilution			
Sample Concentration Ranges	Aliquot from Initial Dilution (mL)	Final Dilution Volumetric Flask Size (mL)	Diluent
0 and 1.0 µg/g	N/A	N/A	N/A
100 µg/g	N/A	N/A	N/A
1,000 µg/g	1	10	diluent 1
10,000 µg/g	1	100	diluent 1

6.4.7 Transfer aliquots of each standard into individual crimp top autosampler vials.

6.5 Analytical Run Sequence and Composition

6.5.1 The typical run list should follow this order

5 replicate injections	system suitability (A1 extracted standard)
2 replicate injections	B1 agreement standard (extracted)
1 injection	extracted blank
≤10 injections	unknown samples
1 injection	check standard (A1 extracted standard)

6.5.2 Repeat last two lines as necessary if more then 10 samples are analyzed. A single replicate of the check standard is analyzed after the last unknown sample in the entire analysis batch.

6.6 Analytical Conditions

Use the GC system described below, adjusting conditions if necessary, to approximate the retention time listed below. Refer to the SOP for Chromatographic System Suitability.

6.6.1 Instrumental

Gas Chromatograph:	Hewlett-Packard, HP6890, or equivalent
Autosampler:	Hewlett-Packard, HP7683, or equivalent
Detector:	Hewlett-Packard, HP5973, or equivalent

6.6.2 Injector

Injection Volume:	2 µL
Rinse Solvent:	Acetonitrile

6.6.3 Inlet

Liner:	Restek, Splitless Quartz, 4 mm ID CAT# 22404
Mode:	Splitless
Gas Type:	Helium
Temperature:	150 °C
Pressure:	10.1 psi
Total Flow:	26.6 mL/min
Purge Flow:	21.5 mL/min at 1.50 min

6.6.4 Column

Column:	Restek, Rtx-1, 15 m x 0.32 mm ID, 1 µm film thickness
Mode:	Constant Flow
Flow:	4.0 mL/min

6.6.5 Oven

Initial Temperature:	70°C
Initial Time:	1.5 minutes
Run Time:	10.17 minutes

Oven Ramp		
Rate	Final Temp.	Final Time
(°C/min.)	(°C)	(min.)
30.0	300	1.0

6.6.6 Auxiliary

Heater:	On
Set Point:	280°C

6.6.7 Mass Selective Detector

Ion Source:	Chemical Ionization (CI) / positive
CI Gas:	Methane
CI Gas Flow Control Setting:	20
Acquisition Mode:	Selective Ion Monitoring (SIM)
Solvent Delay:	3.0 minutes (may be adjusted to be as long as possible without eliminating the first peak of interest)
MS Source:	250°C
MS Quad:	106°C

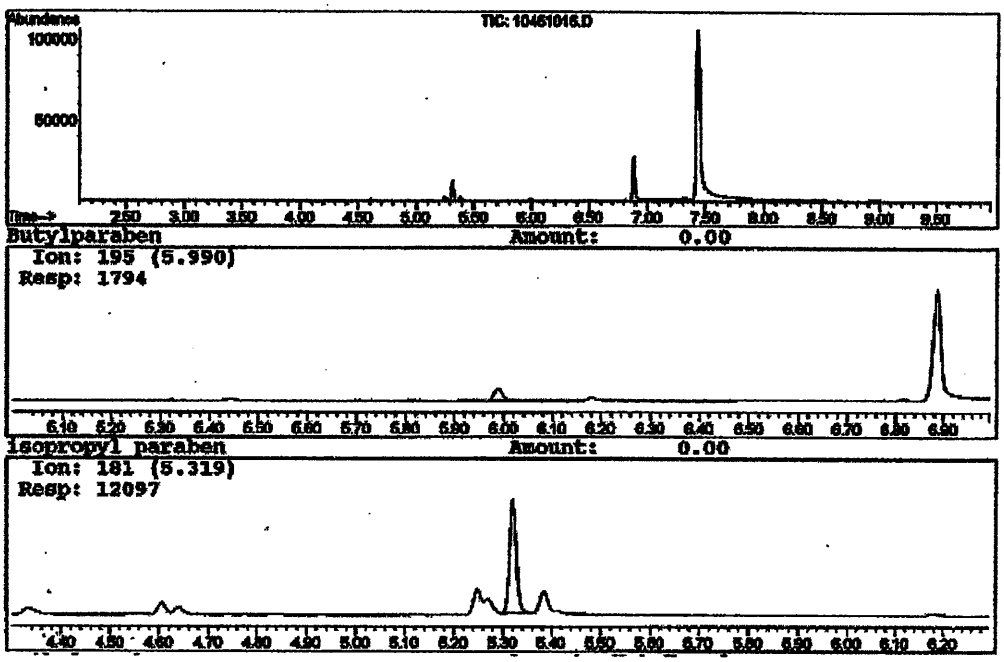
Ions Monitored					
	Start		Nominal	Dwell	Retention
Group	Time[1]	Compound	Ion	Time[2]	Time[3]
Number	(min)	Name	(m/z)	(msec)	(min)
1	3.0	isopropylparaben	181	100	5.3 ± 0.8
		butylparaben	195	100	5.9 ± 0.8

[1] = Start time may be adjusted to maintain analytes within their respective group.

[2] = Dwell times may be modified to optimize performance.

[3] = Approximate retention times (RT) for a new column. Actual RTs may vary as the column is shortened during maintenance.

6.6.8 Example Chromatogram for A1 Standard



6.7 Calculations

6.7.1 Chromatograms will be automatically integrated and visually inspected for an acceptable integration. Manual baselines will be performed when necessary.

6.7.2 Calculate the relative standard deviation (%) of the peak area ratios, the relative standard deviation (%) of the retention time for five system suitability injections. Peak area ratio is defined as: (peak area of butylparaben / peak area of IS).

6.7.3 Calculate the concentration of the A1 calibration standard and the B1 agreement standard from the actual stock concentration, in terms of microgram of butylparaben per milliliter.

6.7.4 Calculate the peak area ratios for the standards and samples relative to the internal standard.

6.7.5 Calculate the response factor for each standard injection by dividing the peak area ratios by the actual standard concentration.

6.7.6 Calculate the mean response factor for the A1 calibration standard (from the 5 system suitability injections) and the mean response factor for the replicate B1 agreement standard injections. Calculate the % difference between the two mean response factors.

6.7.7 Calculate the concentration of unknown samples in µg/g by dividing the peak area ratios by the mean response factor for all calibration standard A1 injections. Correct for the dilution factor if necessary.

6.7.8 Concentrations found to be less than the LOD will be reported as <LOD. Concentrations found to be less than the LLOQ but greater than the LOD will be reported as <LLOQ. In cases, such as blank samples, where no peak is observed, the results will be reported as none detected (N.D.).

6.7.9 Calculate mean concentrations for replicate samples. Calculate the percent error from theoretical as: (mean concentration found – theoretical concentration) / theoretical concentration x 100.

6.8 Acceptance Criteria

6.8.1 System Suitability

The butylparaben peaks in the five system suitability injections must meet the following acceptable limits: The relative standard deviation (%) of the peak area ratios ≤5.0%, and the relative standard deviation (%) of the retention time ≤5.0%. If the criteria are out of the acceptable limits, make corrections to the system and repeat the suitability injections.

6.8.2 Calibration Standard Agreement

The area ratio response factors for the A1 calibration standard and the B1 agreement standard must be within ±5%. If the standards do not agree, prepare a third standard and retest with the outlier of the first set eliminated.

6.8.3 Check Standards

The back- calculated concentration for the A1 check standards must be within 5.0% of nominal theoretical concentration.

6.8.4 Replication of Results

Replicate concentrations found for diet formulations must not vary by more than 15%. Acceptance is defined as: (low value / high value) ≥ 0.85.

Results that do not meet this criteria will be reviewed by the project scientist. Reason for acceptance will be documented in the raw data

6.8.5 Samples

The mean of the back-calculated concentrations for replicate samples must be within ±15.0% of their nominal concentration.

Refer to the Standard Operating Procedure for "Resolution and Reporting of Out of Specification Dose Formulation Analysis Results" if the percent error is greater than ±15.0%.

7 Revision History

7.1 Method validation performed under project PACA-ED00.

7.2 From Revision 00 to Revision 01:

7.2.1 Changed HPLC-UV to GC/MS on page 1.

7.2.2 Corrected the Inlet Pressure from 5.7 psi to 10.1 psi in Section 6.6.3.

7.2.3 Corrected the Purge Flow from 20.0 mL/min to 21.5 mL/min in Section 6.6.3.

7.2.4 Added "x" in Section 6.6.4.

7.2.5 Removed Formulation Stability and Standard Stability from Section 3.

7.3 From Revision 01 to Revision 02:

7.3.1 Section 2: corrected the concentration units for the Final Injected Concentration and the Corresponding Concentrations tables.

7.3.2 Section 3: corrected the concentration units for process stability and added formulation stability data to the table.

7.3.3 Section 4: corrected mg/g to µg/g

7.3.4 Section 6.4.6: corrected the concentration units in the Second Dilution and Final Dilution tables.

7.3.5 Section 6.6.3: corrected typographical error (CAS# was changed to CAT#)

7.3.6 Section 6.7.3: corrected calculated standard concentration units.

7.3.7 Section 6.7.7: specified that unknown sample concentrations should be reported in µg/g.

CHARLES RIVER
LABORATORIES
Discovery and Development Services
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Laboratory Method for the Analysis of Butylparaben in CE-2 Rodent Diet Dose Formulations by HPLC-MS/MS

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Senior Scientist

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Senior Director, Analytical Chemistry Department

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1 Purpose

The purpose of this laboratory method is to accurately determine the concentration of Butylparaben in CE-2 Rodent Diet dose formulations.

2 Scope

Analysis of Butylparaben in dose formulation samples with limitations as stated below.

Vehicle: CE-2 Rodent Diet
Sample Weight (or Amount): 2 grams
Volumetric Samples [ ] Gravimetric Samples [X] Both [ ]

Concentrations Covered by Laboratory Method:

Final Injected Concentration - µg/mL

LOD	0.051
LLOQ to ULOQ	0.8 – 7.0

Corresponding Concentrations - µg/g (ppm) in CE-2 Diet

	Standard Extraction with 1 in 20 Dilution (Total DF[1] = 100)	Additional 1 in 5 Dilution (Total DF[1] = 500)	Additional 1 in 25 Dilution (Total DF[1] = 2500)
LOD	5.1	26	128
LLOQ to ULOQ	80 - 700	400 - 3500	2000 - 17500
Valid Sample Range	0, 92 - 595	460 - 2980	2300 - 14900

[1] DF = Dilution Factor

3 Stability

Description	Concentration Range	Storage Conditions	Time Period
Process Stability	0.8 to 7.0 µg/mL	22 ± 5°C	24 hours
Stability Period 1	100 – 10000 ppm	22 ± 5°C	25 days
Stability Period 2	100 – 10000 ppm	22 ± 5°C	8 weeks

Note: Standards should be prepared fresh for each analysis. All storage conditions are unprotected from light unless specified otherwise.

4 Definitions/Abbreviations

µg/g:	ppm (parts per million)
ACN:	Acetonitrile
APCI:	Atmospheric Pressure Chemical Ionization
HPLC:	High Performance Liquid Chromatography
LLOQ:	Lower Limit of Quantitation
LOD:	Limit of Detection
MPA:	Mobile Phase A
MPB:	Mobile Phase B
MS:	Mass Spectrometry
N/A:	Not applicable
ND:	None detected
ULOQ:	Upper Limit of Quantitation
Purity/Salt Factor:	None – no correction

5 Materials

5.1 Chemicals

Deionized Water, Millipore, Milli-Q water, or equivalent
Acetonitrile, HPLC grade or equivalent
Formic Acid, Reagent grade or equivalent
CE-2 Diet, Meal form from CLEA Japan, Inc.
Isopropylparaben, Sigma, CAS# 4191-7-3-5

5.2 Supplies

Volumetric flasks and pipets
50 mL conical test tubes
Autosampler Vial Caps: Teflon, solid septa, screw top caps or equivalent

6 Procedure

6.1 Preparation of Reagents

Other volumes may be prepared using the same proportions. Store all reagents at room temperature and use within 14 days unless noted otherwise.

6.1.1 Mobile Phase A (0.1% Formic Acid in Water)

Add 1 mL of formic acid to 1000 mL of milli-Q water and mix well.

6.1.2 Mobile Phase B (0.1% Formic Acid in Acetonitrile)

Add 1 mL of formic acid to 1000 mL of acetonitrile and mix well.

6.1.3 Needle Rinse (50:50, ACN: Water, v:v)

Combine 500 mL of milli-Q water with 500 mL of ACN and mix well.

6.1.4 Internal Standard Stock Solution (0.4 mg/mL IS in 90:10 ACN:water, v:v)

Weigh approximately 40 mg of Isopropylparaben into a 100 mL volumetric flask. Add 90 mL of ACN and bring to volume with milli-Q water. Mix well until dissolved. Store refrigerated and use within 90 days of preparation.

6.1.5 Diluent 1 (3 µg/mL IS in Diluent)

A new batch of diluent should be prepared for each analysis. Transfer 15 mL of internal standard stock solution into a 2000 mL volumetric flask. Add 200 mL of milli-Q water and bring to volume with ACN. Mix well. Store refrigerated and use within 7 days.

6.1.6 Blank Feed Extract

A new batch of blank feed extract should be prepared for each analysis. Weigh approximately 20 grams of CE-2 diet into an appropriate container. Add 100 mL of diluent 1 and follow the extraction procedure in section 6.3

6.1.7 Diluent 2 (5% Feed Extract Solution)

A new batch of diluent 2 should be prepared for each analysis. Transfer 25 mL of blank feed extract solution into a 500 mL volumetric flask. Bring to volume with diluent 1 and mix well. Store refrigerated and use within 7 days.

6.2 Preparation of Stocks, Spiking Standards and Blanks

Stocks, spiking standards and blanks should be stored refrigerated.

6.2.1 Preparation of stock solutions

	Butylparaben weight (mg)*	Volumetric Flask (mL)	Diluent
Stock A	20	50	diluent 1
Stock B	25	50	diluent 1

* Record weights to the nearest 0.01 mg.

6.2.2 Preparation of spiking standard solutions

Calibration Standards	Aliquot from Stock A (mL)	Aliquot from Stock B (mL)	Final Volume (mL)	Diluent
A1, A2 and A3	1, 4 and 7	N/A	25	diluent 1
B1, B2 and B3	N/A	2, 4 and 7	25	diluent 1

6.2.3 Preparation of Blank

	Volumetric Flask (mL)	Diluent
Blank	25	diluent 1

6.2.4 Use the spiking standard and blank solutions in section 6.2.2 and 6.2.3 to prepare matrix matched standards (section 6.3).

6.3 Preparation of Matrix-Matched Standards

Store matrix-matched standards refrigerated.

6.3.1 Weigh approximately 2 g of blank CE-2 diet directly into tared 50 mL conical test tubes using a balance capable of reading at least 0.01 g.

6.3.2 Using volumetric pipettes, add 10 mL of each standard and blank spiking solutions to individual tubes containing diet.

6.3.3 Tightly cap the test tubes, vortex each for approximately 5 minutes, tumble on a rotary tumbler for approximately 30 minutes at a speed of 30% and

sonicate for approximately 15 minutes. Centrifuge for 5 minutes at a speed of 3000 rpm.

6.3.4 Remove the supernatant as soon as possible after the completion of the extraction and transfer to a 20 mL scintillation vial.

6.3.5 Using a volumetric pipette, transfer 1 mL of each extracted standard into individual 20 mL volumetric flasks and dilute to volume with diluent 1.

6.4 Sample Extract Preparation

Store diluted samples refrigerated.

6.4.1 Prepare samples in duplicate by weighing approximately 2 g of sample directly into tared 50 mL conical test tubes using a balance capable of reading at least 0.001 g.

6.4.2 Using volumetric pipettes, add 10 mL of diluent 1 to each test tube.

6.4.3 Tightly cap the test tubes, vortex each for approximately 5 minutes, tumble on a rotary tumbler for approximately 30 minutes at a speed of 30% and sonicate for approximately 15 minutes. Centrifuge for 5 minutes at a speed of 3000 rpm.

6.4.4 Remove the supernatant as soon as possible after the completion of the extraction and transfer to a 20 mL scintillation vial.

6.4.5 The initial sample dilutions may be diluted further as indicated in the dilution tables below. Mix well and transfer an aliquot of each final dilution into individual autosampler vials.

Second Dilution			
Sample Concentration Ranges (µg/g)	Sample Size (mL)	Initial Dilution Volumetric Flask Size (mL)	Diluent
0, 92 – 14900	1	20	Diluent 1

Final Dilution			
Sample Concentration Ranges (µg/g)	Aliquot from Initial Dilution (mL)	Final Dilution Volumetric Flask Size (mL)	Diluent
0 and from 92 to 595	N/A	N/A	N/A
From 460 to 2980	1	5	diluent 2
From 2300 to 14900	1	25	diluent 2

6.5 Analytical Run Sequence and Composition

6.5.1 The typical run list should follow this order

5 replicate injections	system suitability (B3 matrix-matched standard)
1 injection each	six point calibration curve
1 injection	blank
[≤ 10 injections	unknown samples
1 injection	check standard (A3 matrix-matched standard)]
1 injection each	six point calibration curve

6.5.2 Repeat last two lines in brackets as necessary if more then 10 samples are analyzed. The six-point calibration curve is re-analyzed (re-injected) after the last unknown sample in the entire analysis batch.

6.6 Analytical Conditions

Use the HPLC system described below, adjusting the solvent ratio if necessary, to approximate the retention time listed below. Refer to the SOP for Chromatographic System Suitability.

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6.6.1 HPLC Conditions

Pump:	Agilent 1100 LC Binary Pump, Model G1312A or equivalent
Autosampler:	Refrigerated HTS PAL CTC Analytics, Leap Technologies or equivalent
Analytical Column:	Phenomenex Hypersil BDS, 3µ, 50 x 4.6mm
Pre-column frit:	Upchurch Scientific, SS frit, 0.094 x 0.25mm
Column Temperature:	ambient
Autosampler Temp:	5°C
Injection Volume:	15 µL
Mobile Phase A:	0.1% Formic Acid in Water
Mobile Phase B:	0.1% Formic Acid in Acetonitrile
Needle Rinse:	50:50 Milli-Q Water:Acetonitrile
Needle Wash 1:	Pre-cleans: 0 Post-cleans: 2 Valve cleans: 2
Needle Wash 2:	Not used
Flow Rate:	1 mL/min
Run Time:	4 minutes
Retention Time:	Butylparaben: 1.8 ± 0.5 minutes Isopropylparaben: 1.6 ± 0.5 minutes
Run Type:	Gradient Program

Total Time (min)	MPA%	MPB%
0	60	40
0.5	60	40
2.0	5	95
3.5	5	95
4.0	60	40

6.6.2 MS/MRM Conditions:

Mass Spectrometer:	PE Sciex, API 300 or equivalent
Interface:	APCI, positive ion mode
Scan Mode:	Multiple Reaction Monitoring (MRM)
Source Temp:	500°C *
Transitions:	m/z 195.1 --> 138-9 – Butylparaben, dwell time = 200 msec * m/z 181.1--> 138.9 – isopropylparaben, dwel time = 200 msec *

* May be optimized to improve analytical run.

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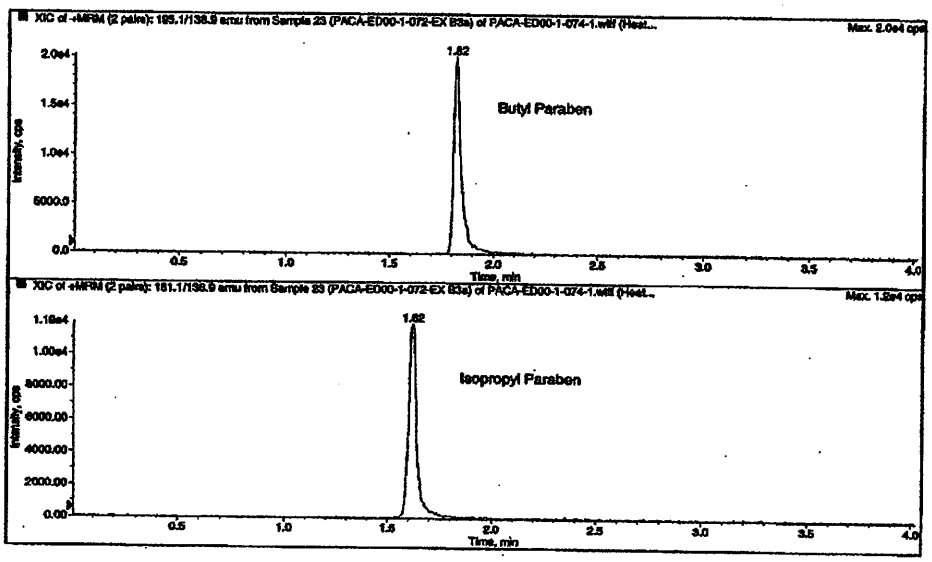
6.6.3 Example Chromatogram for B3 Matrix-Matched Standard



6.7 Calculations

6.7.1 Chromatograms will be automatically integrated and visually inspected for an acceptable integration. Manual baselines will be performed when necessary.

6.7.2 Calculate the relative standard deviation (%) of the peak area ratios, the relative standard deviation (%) of the retention time of Butylparaben and internal standard in five system suitability injections. Calculate the tailing factor of Butylparaben and the internal standard for one system suitability injection.

6.7.3 Calculate the peak area ratios of each injection as follows (response area of butylparaben / response area of internal standard).

6.7.4 Calculate the concentration of the six matrix-matched standards from the actual stock concentration, in terms of micrograms of Butylparaben per milliliter.

6.7.5 Compute the unweighted linear regression relating the peak area ratios of the matrix-matched standards to their respective Butylparaben concentrations, without blank correction.

6.7.6 Compute the correlation coefficient for the standard curve.

6.7.7 Using the peak area ratios of the samples and the regression equation, determine the concentration in µg/g of Butylparaben. Correct for the dilution factor if necessary.

6.7.8 Concentrations found to be less than the LOD will be reported as <LOD. Concentrations found to be less than the LLOQ but greater than the LOD will be reported as <LLOQ. In cases, such as blank samples, where no peak is observed, the results will be reported as none detected (N.D.).

6.7.9 Calculate mean concentrations for replicate samples. Calculate the percent error from theoretical as: (mean concentration found – theoretical concentration) / theoretical concentration x 100.

6.8 Acceptance Criteria

6.8.1 System Suitability

The Butylparaben peaks in the five system suitability injections must meet the following acceptable limits: he relative standard deviation (%) of the peak area ratios ≤5.0% and the relative standard deviation (%) of the retention times for butylparaben and isopropylparaben ≤2.0%. The tailing factor for one system suitability injection should be ≤2.0 If the criteria are out of the acceptable limits, make corrections to the HPLC system and repeat the suitability injections.

6.8.2 Correlation Coefficient

The correlation coefficient for the standard curve must not be less than 0.99. If the value does not exceed 0.99, repeat the preparation of the standard curve.

6.8.3 Calibration Standards

The back-calculated concentrations for calibration standards must be within ±15% of their nominal theoretical concentrations. Standards not meeting criteria can be dropped as long as no more than 20% of standards are dropped. The LLOQ or ULOQ will be redefined to the remaining lowest or highest standards if necessary.

6.8.4 Check Standards

The back- calculated concentration for the A3 check standards must be within 10.0% of nominal theoretical concentration.

6.8.5 Replication of Results

Replicate concentrations found for diet formulations must not vary by more than 15%. Acceptance is defined as: (low value / high value) ≥ 0.85. Results that do not meet this criteria will be reviewed by the project scientist. Reason for acceptance will be documented in the raw data.

6.8.6 Samples

The mean of the back-calculated concentrations for replicate samples must be within ±15.0% of their nominal concentration.

Refer to the Standard Operating Procedure for "Resolution and Reporting of Out of Specification Dose Formulation Analysis Results" if the percent error is greater than ±15.0%.

7 Revision History

7.1 Method validation performed under project PACA-ED00.

7.2 From Revision 00 to 01:

7.2.1 Section 3: Updated stability table.

7.2.2 Section 6.7.2: Corrected typographical error and clarified what should be calculated for system suitability.

7.2.3 Section 6.7.3: Corrected typographical error.

CLEA Rodent Diet CE-2

Components

Table-1.
-Materials-

Protein source: soybean meal, white fish meal
Fat source: soybean oil
Fiber source: alfalfa meal
Carbohydrate source: wheat, corn, wheat bran, germ, leaven, defatted rice bran, milo
Vitamin mixture
Mineral mixture

Table-2.
-Composition and Calorie-(Per 100g diet)

Moisture	(%)	8.9
Crude protein	(%)	25.4
Crude fat	(%)	4.4
Crude fiber	(%)	4.1
Crude ash	(%)	6.9
NFE	(%)	50.3
Calorie*	(kcal)	342.2

*Calories are calculated at 4 protein, 9 fat and 4 NFE.

Table-3.
-Mineral- (Per 100g diet)

Calcium	(g)	1.18
Phosphorus	(g)	1.03
Magnesium	(g)	0.29
Potassium	(g)	1.06
Sodium	(g)	0.26
Manganese	(mg)	10.57
Iron	(mg)	26.0
Copper	(mg)	1.25
Zinc	(mg)	6.38
Cobalt	(mg)	0.13
Iodine	(μg)	45.5
Ca/P		1.15
Ca/Mg		4.07
K/Na		4.08

Table-4.
-Vitamin- (Per 100g diet)

V.A	(IU)	1,517
V.D_3	(IU)	250
V.E	(mg)	7.0
V.B_1	(mg)	1.7
V.B_2	(mg)	1.3
V.B_6	(mg)	1.2
V.B_{12}	(μg)	3.4
V.C	(mg)	19
Pantothenic acid	(mg)	3.7
Niacin	(mg)	16.7
Folic acid	(mg)	0.2
Choline	(mg)	195
Biotin	(μg)	48.4
Inositol	(mg)	549

APPENDIX F

TEMPERATURE AND RELATIVE HUMIDITY REPORTS

ARGUS

Temperature and Relative Humidity Report
Location: Room 11

Protocol Number: 1203-006

Range of Dates: 15-Jun-2004 13:40 to 20-Aug-2004 08:59

	Temperature		Relative Humidity	
Target Range:	64°F to 79°F		30% to 70%	
Species: Rat				
Total Number of Days:	67		67	
Total Number of Hours:	1579.0		1579.0	
Total Number of Data Points:	1573		1573	
Mean (± SD):	69.7	(± 1.3)	60.0	(± 5.0)
Maximum:	72.6		73.7	
Median:	69.3		60.5	
Minimum:	67.0		45.7	
Number of Points in Range (%):	1573	(100.0)	1547	(98.3)
Number of Points High (%):	0	(0.0)	26	(1.7)
Number of Points Low (%):	0	(0.0)	0	(0.0)

Report Generated: 20-Aug-2004 at 12:38

COMMENTS: _____

REVIEWED BY: RKmesho **DATE:** 8.2004

ARGUS

Relative Humidity Deviations Report
Location: Room 11

Protocol Number: 1203-006

Range of Dates: 15-Jun-2004 13:40 to 20-Aug-2004 08:59

Humidity Target Range: 30% to 70%
Species: Rat

Date	Time	R.H.
23-Jul-2004	18:00	72.2 H
23-Jul-2004	20:00	70.7 H
23-Jul-2004	21:00	71.1 H
23-Jul-2004	22:00	70.1 H
24-Jul-2004	01:00	70.5 H
27-Jul-2004	11:00	70.4 H
27-Jul-2004	13:00	70.5 H
27-Jul-2004	14:00	72.8 H
27-Jul-2004	15:00	70.4 H
27-Jul-2004	16:00	73.4 H
27-Jul-2004	17:00	71.7 H
27-Jul-2004	18:00	71.1 H
27-Jul-2004	19:00	70.8 H
27-Jul-2004	20:00	73.0 H
27-Jul-2004	22:00	71.4 H
28-Jul-2004	01:00	70.5 H
28-Jul-2004	02:00	70.2 H
28-Jul-2004	08:00	70.6 H
28-Jul-2004	11:00	71.7 H
30-Jul-2004	01:00	70.5 H
30-Jul-2004	04:00	71.9 H
30-Jul-2004	05:00	70.1 H
30-Jul-2004	06:00	72.9 H
30-Jul-2004	09:00	73.7 H
03-Aug-2004	06:00	70.7 H
03-Aug-2004	09:00	71.1 H

H = Value out of range - High L = Value out of range - Low
R.H. = Relative Humidity (%)

Report Generated: 20-Aug-2004 at 12:41

✓ These deviations did not adversely affect the outcome or interpretation of the study.

_____ The following deviation(s) impacted on the outcome of the study as described:

Study Director: C Hober Date: 8/20/04

APPENDIX G

DIET ANALYSES

Southern Testing & Research Laboratories

A Division of Microbac
3809 Airport Drive NW, Wilson, NC 27896-8649 • 252-237-4175 • FAX: 252-237-9341
www.southerntesting.com

REPORT of ANALYSIS

SAMPLE No.: N9281-002 / AA22916
Date Reported: Monday, October 18, 2004

John Barnett
CRL-DDS Argus Division
905 Sheehy Drive, Building A.
Horsham, PA 19044

Phone / Fax: 215-443-8710/215-443-8587
P. O.: 136214

Client Sample Marks: CLEA CE-2 Lot#E-Z044AQYA

Sample Collection Date:	10/4/2004	12:00:00 AM	Matrix:	Feed
Lab Submittal Date:	9/10/2004	10:06:00 AM	Classification:	F

CAT No.	ANALYSES	METHOD	ANALYZED	by	RESULT	UNIT
FO-046	PABA		10/18/2004	SW	6.92	mcg/g

Sample Comments:

Reviewed and Approved by:

Shelia Hinnant
Manager, Food Sciences Department

Admin 10/18/2004 10:07:07 AM

Southern Testing & Research Laboratories

A Division of Microbac
3809 Airport Drive NW, Wilson, NC 27896-8649 • 252-237-4175 • FAX: 252-237-9341
www.southerntesting.com

REPORT of ANALYSIS

SAMPLE No.: N9281-003 / AA22917
Date Reported: Monday, October 18, 2004

John Barnett
CRL-DDS Argus Division
905 Sheehy Drive, Building A.
Horsham, PA 19044

Phone / Fax: 215-443-8710/215-443-8587
P. O.: 136214

Client Sample Marks: CLEA CE-2 Lot#E-Z034-YA

Sample Collection Date:	10/4/2004	12:00:00 AM	Matrix:	Feed
Lab Submittal Date:	9/10/2004	10:06:00 AM	Classification:	F

CAT No.	ANALYSES	METHOD	ANALYZED	by	RESULT	UNIT
FO-046	PABA		10/18/2004	SW	4.37	mcg/g

Sample Comments:

Reviewed and Approved by:

Shelia Hinnant
Manager, Food Sciences Department

Admin 10/18/2004 10:07:07 AM

Southern Testing & Research Laboratories

A Division of Microbac
3809 Airport Drive NW, Wilson, NC 27896-8649 • 252-237-4175 • FAX: 252-237-9341
www.southerntesting.com

Lab Sample #: P0513-001-004
Received from:
John Barnett, Sr.
CR-DDS Argus Division
905 Sheehy Drive
Horsham, PA 19044-1241

Report Date: 11/19/04
Received: 10/28/04

Phone#: 215-443-8710
Fax#: 215-443-8587

Method Reference: INA Method 118.000

Results of Analysis

STRL#	Client Marks	Isoflavones, Total (µg/g)
P0513-001	CEA-CEZ Lot#E-205476	773
P0513-002	CEA-CEZ Lot#E-2044A2YA	912
P0513-003	CEA-CEZ Lot#E-2034YA	924

Note-Total Isoflavones are the summation of the following phytoestrogens:
Diadzin, Glycitin, Genistin, Daidzein, Glycitein and Genistein

Reviewed and Approved by:

Shelia Hinnant
Manager, Food Chemistry Dept.

APPENDIX H

HISTOPATHOLOGY REPORT

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

CHARLES RIVER LABORATORIES, DISCOVERY AND
DEVELOPMENT SERVICES, ARGUS DIVISION
STUDY NUMBER 1203-006
EPL PROJECT NUMBER 601-005

ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

PATHOLOGY REPORT

Submitted by:

Experimental Pathology Laboratories, Inc.
P.O. Box 474
Herndon, VA 20172-0474
(703) 471-7060

Submitted to:

Charles River Laboratories, Discovery and
Development Services, Argus Division
Horsham, PA 19044-1241

March 16, 2005

FINAL REPORT

EPL®

EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

TABLE OF CONTENTS

PATHOLOGY SUMMARY

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

CHARLES RIVER LABORATORIES,
DISCOVERY AND DEVELOPMENT
SERVICES, ARGUS DIVISION
STUDY NUMBER 1203-006
EPL PROJECT NUMBER 601-005

ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

PATHOLOGY SUMMARY

Light microscopic examination was performed on tissues from rats that received butylparaben, administered in the diet for at least 56 days beginning on day 21 postpartum. The purpose of the study was to test for toxic effects/ disturbances resulting from oral (diet) exposure to butylparaben of Crl: (WI) BR male rats on spermatogenesis. The study design was as follows:

Dosage Group	Number of Rats	Concentration (ppm)
I	16	0
II	16	100
III	16	1000
IV	16	10000

The test substance was considered 100% pure for the purpose of dosage calculations.

MATERIALS AND METHODS

A necropsy was performed by the Sponsor, Charles River Laboratories, Discovery and Development Services, Argus Division. Protocol-required tissues were collected and fixed in 10% neutral buffered formalin except for the right testis, which was fixed in modified Davidson's Solution for 24 to 48 hours and then retained in 10% neutral buffered formalin. The fixed tissues from all rats were shipped to Experimental Pathology Laboratories, Inc. (EPL®) and were

trimmed, processed, embedded in paraffin, microtomed, placed on glass microscope slides, and stained with hematoxylin and eosin. An additional section of testes was stained with Periodic-Acid Schiff. The following protocol-required tissues were evaluated, as available, from all animals in Groups I and IV: epididymis (remaining portion of left epididymis and right epididymis), prostate, seminal vesicles and testis (right). Adrenal, liver, pituitary and thyroid glands were evaluated from five Group I and six Group IV rats. The microscopic examination was conducted by Peter C. Mann, DVM, Diplomate, ACVP.

Following the initial evaluation of the testes, a detailed qualitative examination of the testes was completed in order to identify treatment-related effects such as missing germ cell layers or types, retained spermatids, multinucleate or apoptotic germ cells and sloughing of spermatogenic cells into the lumen. Any cell- or stage-specific testicular findings were noted. A semi-quantitative staging of the testes was completed for each PAS-stained section of testes. Twenty-five cross sections of seminiferous tubules were evaluated from each animal. The tubules were grouped as follows, using a modified version of the staging criteria described in Russell et al, 1990, and the Binary Decision Key in Hess, 1990.

Stages I-III: There are two generations of spermatids. The elongated spermatids do not line the lumen. The acrosomic system over the nucleus is not developed.

Stages IV-VI: There are two generations of spermatids. The elongated spermatids do not line the lumen. The acrosomic system over the nucleus is developed.

Stages VII-VIII: There are two generations of spermatids. The elongated spermatids line the lumen. The acrosomic system over the nucleus is developed.

Stage IX: There is one generation of spermatids. The mature spermatids are missing from the lumen. Residual bodies are present, and the acrosomes extend the full length of the slightly flattened nucleus.

Stages X-XIII: There is one generation of spermatids. The nuclei become progressively thinner and elongated.

Stages XIV: There is one generation of spermatids. Meiotic figures are present.

Microscopic findings for each animal are listed in the Histopathology Incidence Tables and are graded one to five depending on severity. All findings for all animals are summarized by treatment group in the Summary Incidence Tables, together with the total number of animals in each group. The results for the semi-quantitative staging are presented in Appendix A.

GROSS LESIONS

There were no treatment-related macroscopic lesions present in Groups I and IV.

MICROSCOPIC EXAMINAT ION

There were no treatment-related lesions present in the male reproductive system or in the adrenal, liver, pituitary or thyroid glands for those animals examined. One rat given 10000 ppm butylparaben had a single cross section of a seminiferous tubule with degeneration characterized by loss of germinal epithelium. Because of the extremely small area affected and the fact that this was the only lesion present in the testes of any animal in the study, this change was not considered treatment-related.

The semi-quantitative staging of the testes did not reveal any cell- or stage-related changes in the testes of either control or treated animals. The number of

tubules in the stage groups (Appendix A) was similar for control and treated animals.

CONCLUSION

Administration of butylparaben to male rats, administered in the diet for at least 56 days beginning on Day 21 postpartum did not result in macroscopic or microscopic treatment-related changes in the male reproductive tract (testes, epididymis, prostate, seminal vesicles) or in the adrenal, liver, pituitary or thyroid glands.

Peter C. Mann March 16, 2005

PETER C. MANN, DVM, Diplomate, ACVP
Veterinary Pathologist

Date

PCM/cb

REFERENCES

Hess, R. A. (1990). Quantitative and Qualitative Characteristics of the Stages and Transitions in the Cycle of the Rat Seminiferous Epithelium: Light Microscopic Observations of Perfusion-Fixed and Plastic Embedded Testes. Biology of Reproduction. **43**:525-542.

Russell, L. D., Ettlin R. A., Sinha Hikim, A. P., Clegg, E. D. (1990). Histological and Histopathological Evaluation of the Testis. Cache River Press, Clearwater, FL.

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

COMPLIANCE STATEMENT

Client Name	Charles River Laboratories, Discovery and Development Services, Argus Division	EPL Project Coordinator	Kristi Larson
Client Study	1203-006	EPL Pathologist	Dr. Peter Mann
Species	Rat	EPL Project Number	601-005
Study Title	Oral (Diet) Reproduction Toxicity Study of Butylparaben in Male Rats		
Test Article	Butylparaben		

The Histopathology portions of the above-referenced study were conducted in compliance with the Good Laboratory Practice regulations of the Food and Drug Administration as stipulated by 21 CFR Part 58.

Kristi T. Larson
EPL Project Coordinator

3-17-05
Date

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EXPERIMENTAL PATHOLOGY LABORATORIES, INC.

QUALITY ASSURANCE FINAL CERTIFICATION

Study Title: Oral (Diet) Reproduction Toxicity Study of Butylparaben in Male Rats

Client Study: 1203-006 EPL Project Coordinator: Kristi Larson

EPL Project Number: 601-005 EPL Pathologist: Dr. Peter Mann

The following aspects of this study were inspected by the Quality Assurance Unit of Experimental Pathology Laboratories, Inc. Dates inspections were performed and findings reported to the EPL Project Coordinator and Management are indicated below.

Area Inspected	Dates	
	Inspection	Reporting
EPL Project Sheets	8/17/04	8/17/04
Project Setup	9/2,8/04	9/2,8/04
Histology Setup	9/9/04	9/9/04
In-Process	9/13/04	9/13/04
Data Review	9/29/04	9/29/04
Draft Report	10/20/04	10/20/04
Final Report	3/17/05	3/17/05

Date reported to Study Director/Management 3/17/05

Date of last quarterly facility inspection 1/05

Jan S. Snyder 3/17/05

EPL Quality Assurance Unit Date

Form No. 6-2 (7/2/99)

SUMMARY INCIDENCE TABLES

SUMMARY INCIDENCE TABLE

1203-006
Terminal Sacrifice
Male Rat

	GROUP I	GROUP II	GROUP III	GROUP IV		
ADRENAL (NO. EXAMINED)	(5)			(6)		
EPIDIDYMIS (NO. EXAMINED)	(16)			(16)		
Immature Spermatids	1					
Infiltration, Mononuclear						
Cell, Focal				4		
EYE (NO. EXAMINED)	(1)	(1)				
Hemorrhage, Periorbital		1				
Panopthalmitis, Acute	1					
LIVER (NO. EXAMINED)	(5)			(6)		
Infiltration, Mixed Cell,						
Focal	1					
Infiltration, Mononuclear						
Cell, Focal	3			5		
PITUITARY (NO. EXAMINED)	(4)			(6)		
PROSTATE (NO. EXAMINED)	(16)			(16)		
Infiltration, Mononuclear						
Cell, Focal	3			3		
SEMINAL VESICLE (NO. EXAMINED)	(16)			(16)		
TESTIS, RIGHT (NO. EXAMINED)	(16)			(16)		
Degeneration, Seminiferous						
Tubule, Focal				1		
THYROID (NO. EXAMINED)	(4)			(6)		

HISTOPATHOLOGY INCIDENCE TABLES

HISTOPATHOLOGY INCIDENCE TABLE

1203-006 Terminal Sacrifice Male Rat	GROUP I																		GROUP II		
ANIMAL	12737	12738	12739	12740	1u2741	12742	12743	12744	12745	12746	12747	12748	12749	12750	12751	12752		1u2753			
ADRENAL	X	X	N									N	X	X		X					
EPIDIDYMIS	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X					
Immature Spermatids															1						
Infiltration, Mononuclear																					
Cell, Focal																					
EYE																					
Hemorrhage, Periorbital																		3			
Panopthalmitis, Acute					4																
LIVER			N									N				X					
Infiltration, Mixed Cell,																					
Focal														1							
Infiltration, Mononuclear																					
Cell, Focal	1	1											1								
PITUITARY	N	X	N									N	X	X		X					
PROSTATE	X	X		X	X	X	X	X	X	X	X	X	X	X							
Infiltration, Mononuclear																					
Cell, Focal			1												1	1					
SEMINAL VESICLE	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
TESTIS, RIGHT	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Degeneration, Seminiferous																					
Tubule, Focal																					
THYROID	N	X	N									N	X	X		X					

Key: X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

HISTOPATHOLOGY INCIDENCE TABLE

1203-006 Terminal Sacrifice Male Rat		GROUP IV																			
	ANIMAL	12785	12786	12787	12788	12789	12790	12791	12792	12793	12794	12795	12796	12797	12798	12799	12800				
ADRENAL		X		X	X			X				X					X				
EPIDIDYMIS		X	X	X		X		X	X	X	X	X		X		X	X				
Immature Spermatids																					
Infiltration, Mononuclear																					
Cell, Focal					1		1						1		1						
LIVER								X													
Infiltration, Mixed Cell,																					
Focal																					
Infiltration, Mononuclear																					
Cell, Focal		1		1	1							1					1				
PITUITARY		X		X	X			X				X					X				
PROSTATE		X	X	X	X	X		X	X			X	X	X	X	X	X				
Infiltration, Mononuclear																					
Cell, Focal							1			1	1										
SEMINAL VESICLE		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
TESTIS, RIGHT		X	X	X	X	X	X	X	X	X	X		X	X	X	X	X				
Degeneration, Seminiferous																					
Tubule, Focal												1									
THYROID		X		X	X			X				X					X				

Key: X=Not Remarkable N=No Section I=Incomplete A=Autolysis
1=minimal 2=slight/mild 3=moderate 4=moderately severe 5=severe/high
P=Present B=Benign M=Malignant
m=missing one paired organ u=unscheduled sac./death

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

1203-006
Terminal Sacrifice

Species: Rat Sex: Males Group Identification: I - Unscheduled Sac./Death

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
12741	EYE	Exophthalmos	Panopthalmitis, Acute
	EYE	Traumatized cornea	Panopthalmitis, Acute
	*	Swollen head: right side	Panopthalmitis, Acute (EYE)

CORRELATION OF GROSS AND MICROSCOPIC FINDINGS

1203-006
Terminal Sacrifice

Species: Rat Sex: Males Group Identification: II - Unscheduled Sac./Death

Animal Number	Client Topography / Site	Client Gross Observations	Microscopic Observations
12753	EYE	Exophthalmos	Hemorrhage, Periorbital
	*	Swelling-head periorbital	Hemorrhage, Periorbital (EYE)

APPENDIX A
SEMIQUANTITATIVE STAGING OF TESTES

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Charles River Laboratories, Discovery and Development Services,
Argus Division Study Number 1203-006

Appendix A: Semiquantitative Staging of Testes

	I-III	IV-VI	VII-VIII	IX	X-XIII	XIV
12737	7	2	6	2	9	
12738	5	4	8	2	6	
12739	6	3	8	1	5	2
12740	5	4	7	1	8	
12741	6	4	8	3	3	1
12742	4	4	6		10	1
12743	4	3	9	1	8	
12744	6	2	4	1	10	2
12745	2	4	8	1	9	1
12746	5	3	8		8	1
12747	6	3	11	1	4	
12748	5	5	6	1	6	2
12749	4	4	6	3	7	1
12750	4	2	6	1	12	
12751	4	2	9		10	
12752	5	3	6	2	9	
12785	4	4	10		6	1
12786	6	2	6	2	7	2
12787	2	1	8		12	2
12788	4	3	8	1	8	1
12789	3	4	8	2	6	2
12790	2	4	8		11	
12791	2	1	5	1	14	2
12792	5	2	5	2	9	2
12793	4	2	8	3	8	
12794	4	4	3		13	1
12795	7	4	6	1	7	
12796	5	5	7		7	1
12797	1	4	9	2	8	1
12798	6	2	7		10	
12799	7	1	7	1	8	1
12800	6	1	4	3	10	1

APPENDIX I

STATEMENT OF THE STUDY DIRECTOR

CHARLES RIVER
LABORATORIES
Discovery and Development Services
Argus Division

PROTOCOL 1203-006 - ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

STATEMENT OF THE STUDY DIRECTOR

This final report accurately reflects the raw data obtained during the performance of the study. No deviations from the U.S. Food and Drug Administration (FDA) Good Laboratory Practice Regulations (GLP); Final Rule[a] occurred that affected the quality or integrity of the study with the following exceptions:

1. the bulk chemical is a commercially available product and therefore the Certificate of Analysis (C of A) provided with the chemical was not prepared in compliance with GLP;
2. the bulk chemical was not analyzed after completion of the study because the C of A documented the stability of the bulk chemical through February 2006; and
3. the analyses of the diets for total parabens and phytoestrogens were not conducted in compliance with GLP.

None of these exceptions affected the outcome of the study.

17 MAR 2006

Alan M. Hoberman, Ph.D., DABT Date
Director of Research
and Study Director
CR-DDS Argus Division

---

a. U.S. Food and Drug Administration. Good Laboratory Practice Regulations; Final Rule. 21 CFR Part 58.

APPENDIX J

QUALITY ASSURANCE STATEMENT

CHARLES RIVER
LABORATORIES
Discovery and Development Services
Argus Division

QUALITY ASSURANCE STATEMENT

Argus Protocol: 1203-006

This study has been inspected by the Quality Assurance Unit to assure conformance with the Good Laboratory Practice (GLP) regulations promulgated by U.S. Food and Drug Administration. Reports were submitted in accordance with Standard Operating Procedures as follows:

QA INSPECTION DATES

		Dates Findings Submitted to:	
Dates of Inspection	Phase(s) Inspected	Study Director	Study Director Management
10 JUN 04	Protocol	10 JUN 04	10 JUN 04
22 JUN 04	Sacrifice	28 JUN 04	28 JUN 04
06 JUL 04	Blood Collection	12 JUL 04	12 JUL 04
19 JUL 04	Test Article Administration	02 AUG 04	02 AUG 04
18 AUG 04	Terminal Blood Collection	18 AUG 04	18 AUG 04
18 AUG 04	Sacrifice/Sperm Evaluation	19 AUG 04	19 AUG 04
20 AUG 04	Test Article Preparation Raw Data Check	20 AUG 04	20 AUG 04
09 NOV 04	Hormone Analysis	11 NOV 04	11 NOV 04
08, 12-13 OCT 04	Formulation Data	15 OCT 04	15 OCT 04
13-15 OCT 04	In-Life Data	15 OCT 04	15 OCT 04
15 OCT 04	Necropsy	15 OCT 04	15 OCT 04
15-19, 26, 29 NOV 04	Hormone Assay Data	29 NOV 04	29 NOV 04
28-30 DEC 04	Hormone Assay Calculations	30 DEC 04	30 DEC 04
27-28 OCT 04 01 NOV 04, 30 DEC 04	Report Tables	01 NOV 04, 30 DEC 04	01 NOV 04, 30 DEC 04
14 NOV 04	Results and Summary	15 NOV 04	15 NOV 04
16-17 NOV 04	Methods	17 NOV 04	17 NOV 04
21 JAN 05 15 MAR 05 17 MAR 05	Revised Report	21 JAN 05 15 MAR 05 17 MAR 05	21 JAN 05 15 MAR 05 17 MAR 05

905 Sheehy Drive, Bldg. A, Horsham, PA 19044 • 215.443.8710 • FAX 215.443.8587

The final report has been reviewed to assure that it accurately describes the materials and methods, and the reported results accurately reflect the raw data.

3.17.05

Steven J. Schnell
Quality Assurance Auditor
Principal Auditor

Date

905 Sheehy Drive, Bldg. A, Horsham, PA 19044 • 215.443.8710 • FAX 215.443.8587

Comparison of Oishi (2001) Butylparaben Study With the Charles River-Argus (2005) Butylparaben Study

Endpoint	**Oishi (2001) Study**	**Charles River - Argus (2005) Study**
PROTOCOL		
Rat strain/source	male Crj:Wistar/Charles River, Japan	male Crl:Wistar/Charles River, North Carolina
Housing	individually housed in wire-bottom stainless steel cages, 21-25^{0}C, RH 55 ± 5%, 12-hour light-dark cycle	individually housed in wire-bottom stainless steel cages, 19-23^{0}C, RH 70-74%, 12-hour light-dark cycle
Diet	CE-2 feed, Clea, Tokyo Japan isoflavone levels reported in mouse study Oishi (2002) - which were originally reported in a bisphenol A paper	CE-2 feed, Clea, Tokyo Japan diet analyzed for isoflavones and p-aminobenzoic acid
Age/Weight at start of treatment	19-21 days/49.9 ± 3.01 g	21 days/28.3-48.7 g
Number of male rats per treatment group	8	16
Dietary concentrations of Butylparaben	0, 0.01%, 0.1%, 1.0% (0, 100, 1000, 10000 ppm)	0, 100, 1000, 10000 ppm
Duration of treatment	8 weeks	56 days (8 weeks)
In-life measurements	body weight (daily) food consumption (daily)	body weight (daily) food consumption (2x/week) week 3, 5, 7 orbital sinus blood draw for measurement of testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) blood samples were collected at same time of day each week

Necropsy	organ weights: testes, epididymides, ventral prostates, preputial glands, seminal vesicles with coagulation gland	organ weights: liver, adrenal glands (paired), thyroid, pituitary, right testis, left testis, left epididymis (whole and cauda), right epididymis, seminal vesicles (with and without fluid), prostate (ventral and dorsal)
Sperm counts/Daily sperm production (DSP)	right testis	left testis
Sperm motility/concentration/ morphology	motility/morphology not determined. Concentration of sperm in cauda epididymides determined	computer-assisted sperm analysis (CASA) used; motility assessed from sample from the left vas deferens; concentration and morphology of sperm from left cauda epididymis
Hormone analysis	testosterone (kit from Oxford Biomedical Research)	ELISA methodology used to measure all hormones testosterone (kit Biomedia), LH (kit Amersham Pharmacia Biotech), FSH (Amersham Pharmacia Biotech kit)
Histopathology reproductive organs	not completed	remaining left epididymis, right epididymis, right testis, prostate, seminal vesicles control and high concentration groups, testicular staging was also completed
RESULTS		
Body weights at study termination	no significant differences 0 396 ± 34.5 100 393 ± 21.5 1000 400 ± 19.9 10000 378 ± 19.0	no significant differences 0 300.8 ± 32.0 100 294.1 ± 31.6 1000 312.5 ± 33.5 10000 301.2 ± 31.4

Mortality/early sacrifice	no premature deaths reported	2 rats (1 at 0 ppm, 1 at 100 ppm) were sacrificed because of eye lesions from retro-orbital bleeding
Consumed doses (means)	10.4, 103, 1026 mg/kg/day	10.9, 109.3, 1087.6 mg/kg/day
Isoflavone levels	in µg/g diet daidzin 125, glycirin 26.7, genistin 146, daidzein 3.95, glycitein 25.4, genistein 3.87 (total = 330.92)	in 2 lots of diet 912, and 924 µg/g diet; p-aminobenzoic acid 6.92 and 4.37 ppm
Reproductive organ weights	no effects on testes, ventral prostates, preputial glands absolute weights of the epididymis and seminal vesicles with coagulation glands were significantly lower at 10000 ppm relative weights of epididymis decreased in dose-dependent manner - significant at 1000 ppm at above Relative epididymides weights 0 0.267 ± 0.0191 100 0.247 ± 0.0162 1000 0.237 ± 0.134 10000 0.233 ± 0.0127	no significant effects weights of left epididymis, cauda epididymis and right epididymis measured separately
DSP	significantly ($p<0.05$) decreased at all doses 0 40.0 ± 5.86 100 33.3 ± 4.9 1000 28.3 ± 2.71 10000 24.5 ± 5.43	no effects 0 36.82 ± 11.38 100 31.81 ± 13.8 1000 31.09 ± 12.53 10000 32.83 ± 15.37

Sperm counts in the cauda epipdydimis	significantly decreased at all doses 0 56.0 ± 12.9 100 41.4 ± 8.22 1000 41.6 ± 9.33 10000 32.6 ± 9.80	no effects 0 49.7 ± 25.0 100 78.3 ± 75.8 1000 56.4 ± 28.1 10000 49.4 ± 16.8
Sperm morphology/motility	not measured	no effects
Hormone analysis	testosterone dose-dependent decrease that was statistically significant at the 2 highest dose Values (ng/ml) estimated from Figure 2 0 9 100 7.5 1000 6 10000 3 (control values from Oishi's propylparaben study 9.08 ± 2.12 ng/ml; control values from Oishi's methyl-, ethylparaben study 11.9 ± 2.08 ng/ml)	no biologically important differences that could be related to treatment were observed; some statistically significant observations were noted - reductions in testosterone at 1000 and 10000 ppm after 3 weeks of exposure, significant increase in testosterone at 10000 ppm after 9 weeks of exposure; reductions in LH at 5 weeks of exposure at 100 and 10000 ppm which were not dose-related Values (ng/ml) at week 9 0 1.085 ± 0.430 100 1.485 ± 0.767 1000 1.031 ± 0.575 10000 1.86 ± 0.968 ($p<0.01$)
Histopathology	not completed	no adverse findings in reproductive organs

TRADE SECRET

Study Title

Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin

TEST GUIDELINES: OECD Guideline for the Testing of Chemicals. Draft New Guideline 428: Skin Absorption: *in vitro* Method (2002)

OECD Draft Guidance Document for the Conduct of Skin Absorption Studies. OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28 (2002)

AUTHOR: William J. Fasano, Sr., B.S.

STUDY COMPLETED ON: November 22, 2004

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
Haskell[SM] Laboratory for Health and Environmental Sciences
Elkton Road, P.O. Box 50
Newark, Delaware 19714-0050

LABORATORY PROJECT ID: DuPont-13966

WORK REQUEST NUMBER: 14807

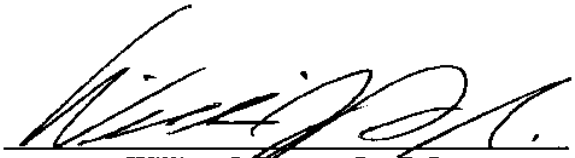
SERVICE CODE NUMBER: 1377

SPONSOR: Cosmetic, Toiletry, and Fragrance Association (CTFA)
Washington, DC
U.S.A.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. FDA (21 CFR part 58) Good Laboratory Practice Standards, which are compatible with the OECD Principles of Good Laboratory Practice (as revised 1997), ENV/MC/CHEM(98)17, OECD, Paris, 1998, and MAFF Japan Good Laboratory Practice Standards (11 NohSan Number 6283) except for the item documented below. The item listed does not impact the validity of the study.

The formulation vehicles used to prepare the oil-in-water emulsions, designated Phases A, B, and C, were not characterized by the sponsor. However, the final emulsion preparations were analyzed for concentration of methylparaben and butylparaben, which were comparable to the target concentrations.

Study Director:  22-Nov-2004

William J. Pasano, Sr., B.S. Date
Research Toxicologist

QUALITY ASSURANCE DOCUMENTATION

Work Request Number: 14807
Study Code Number: 1377

The conduct of this study has been subjected to periodic Quality Assurance inspections. The dates of inspection are indicated below.

Phase Audited	***Audit Dates***	***Dates Reported to Study Director and Management***
Conduct:	April 6, 7, 28, 2004; May 5, 7, 19, 2004; June 11, 2004	April 7, 28, 2004; May 5, 19, 2004; June 11, 2004
Report/Records:	September 1, 2, 7-10, 14-16, 2004 November 4, 2004	September 16, 2004 November 4, 2004

Reported by: Joseph C Hamill 17-Nov-2004
Joseph C. Hamill
Quality Assurance Auditor
Date

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Mass Spectroscopist: _____ 17-Nov-2004
Timothy A. Snow, Ph.D. Date
Senior Research Chemist

Approved by: _____ 17-Nov-2004
Gary W. Jepson, Ph.D. Date
Research Manager

Issued by Study Director: _____ 22-Nov-2004
William J. Fasano, Sr., B.S. Date
Research Toxicologist

This report is approved by the sponsor.

Approved by: Linda Loretz 11-18-04
Linda Loretz **Date**
CTFA Representative

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STUDY INFORMATION

Test Item 1

Substance Tested: Methylparaben

Synonyms/Codes:
- Methyl Paraben
- M3146 (Lot No.)

CAS Registry Number: 99-76-3

Physical Characteristics: White powder

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Test Item 2

Substance Tested: Butylparaben

Synonyms/Codes:
- Butyl Paraben
- B3140 (Lot No.)

CAS Registry Number: 94-26-8

Physical Characteristics: White crystalline powder

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Study Initiated/Completed: December 9, 2003 / (see report cover page)

Experimental Start/Completion: March 16, 2004 / June 24, 2004

SUMMARY

The penetration kinetics and first-pass metabolism of methylparaben and butylparaben in viable rat and human skin has been determined. The active ingredients were formulated as oil-in-water emulsions at a target concentration of 0.8% and 0.4% for methylparaben and butylparaben, respectively. Samples of fresh rat and human skin were dermatomed to approximately 450 μm and mounted *stratum corneum* uppermost, onto a flow-through diffusion cell system with an exposure area of 0.64 cm^2. The underside of each skin specimen was perfused with sterile-filtered Hepes-buffered Hanks' balanced salt solution containing gentimicin (0.05 mg/mL) and bovine serum albumin (3.75%). Each formulated emulsion was applied as a finite dose at a rate of 10 $\mu L/cm^2$ to rat (n = 10 replicates) and human skins (n = 13 replicates). Penetration was followed using [^{14}C]-labeled active ingredient, which was uniformly blended into the emulsions. The amount of active applied per area of skin was approximately 65 $\mu g/cm^2$ and 36 $\mu g/cm^2$ for the methylparaben and butylparaben emulsions, respectively. The applied formulation remained in contact with the skins for 24 hours without occlusion. During the 24-hour exposure period, serial receptor fluid samples were collected hourly for the first 6 hours and then every other hour until termination. At the end of the exposure period, the skin surface was washed with a dilute soap solution to remove excess formulation and then tape-stripped to remove the *stratum corneum*. Distribution of the applied radiolabeled material was determined by liquid scintillation counting. First-pass metabolism was determined by quantitative analysis of serial receptor fluid samples for methylparaben or butylparaben, and the principal metabolite 4-hydroxybenzoic acid using HPLC-mass spectrometry. Receptor fluid samples representing each formulation, from both rat and human skin experiments, were also analyzed by radiochromatography and LC-MS.

A. Methylparaben, 0.8% Oil-in-Water Emulsion

Following application of a 0.8% methylparaben emulsion to viable rat and human skin, a greater amount of total radioactivity penetrated human skin (79.36%) compared to rat skin (54.94%). A major portion of the total radioactivity that had penetrated rat skin was metabolized to 4-hydroxybenzoic acid (53.9%), with a smaller portion (23.8%) accounted for as unmetabolized methylparaben. By comparison, a lesser portion of the total radioactivity that had penetrated viable human skin had been metabolized to 4-hydroxybenzoic acid (35.1%), with the majority (60.3%) accounted for as unmetabolized methylparaben. Exclusive of species, analysis of receptor fluid pools by radiochromatography and LC-MS revealed the presence of methylparaben, 4-hydroxybenzoic acid, and ethylparaben.

B. Butylparaben, 0.4% Oil-in-Water Emulsion

Following application of a 0.4% butylparaben emulsion to viable rat and human skin, a greater amount of total radioactivity penetrated human skin (73.51%) compared to rat skin (54.23%). A majority of the total radioactivity that had penetrated rat skin was metabolized to the primary acid metabolite 4-hydroxybenzoic acid (52.3%), with a smaller portion (5.51%) accounted for as

unmetabolized butylparaben. For human skin, 32.8% of the total radioactivity in the receptor fluid had been metabolized to 4-hydroxybenzoic acid, with a greater portion (49.7%) accounted for as unmetabolized butylparaben. As was observed for the methylparaben formulation, analysis of receptor fluid pools by radiochromatography and LC-MS revealed the presence of butylparaben, 4-hydroxybenzoic acid, and ethylparaben.

Overall these data show, based on a dermatomed viable rat and human skin model and analysis of receptor fluid samples, that the dermal bioavailability of methylparaben and butylparaben from the oil-in-water emulsions was greater for human skin than rat skin, and that a small portion of the total radioactivity that had penetrated the skin, exclusive of species and formulation, was ethylparaben.

INTRODUCTION

Methylparaben and butylparaben are alkyl esters of p-hydroxybenzoic acid and are used as antimicrobial agents in cosmetic and pharmaceutical formulations which may be applied to the skin. The antimicrobial activity of parabens has been shown to increase with an increase in the alkyl ester chain length, which is accompanied by a decrease in water solubility. In the presence of ethanol, an acceptable solvent often used in formulations containing methylparaben or butylparaben, formation of ethylparaben via transesterification can occur.[1] Given that skin contact can represent a major route of exposure to humans, it is important to evaluate the penetration and metabolism of paraben esters following topical application.

The penetration and metabolism of topically applied chemicals and formulations can be determined using an *in vitro* flow-through diffusion cell model.[2-3] Skin samples are obtained fresh, maintained on tissue culture media, trimmed of excess subcutaneous fat, and dermatomed to a level at or about the viable epidermis. Dermatoming skin to the level of the capillaries in the upper region of the dermis helps to prevent retention of hydrophobic chemicals within the hydrophilic dermis. Following preparation, the skin is then mounted *stratum corneum* uppermost onto the receptor chamber, the donor compartment is then clamped in place, and the *in vitro* model equilibrated at 32°C. Following a skin integrity confirmation, the test material is applied to the skin surface and serial receptor fluid samples collected to establish the penetration rate and extent of first-pass metabolism. Skin viability is maintained by perfusing the underside of the membrane with sterile-filtered tissue culture media containing an antibiotic, along with a surfactant such as bovine serum albumin, to enhance the sink conditions for water-insoluble compounds like methylparaben and butylparaben.

The objective of this experiment was to determine the comparative penetration rate and first-pass metabolism of methylparaben and butylparaben to its principal metabolite 4-hydroxybenzoic acid, from an oil-in-water emulsion in viable rat and human skin, by analysis of receptor fluid samples collected from an *in vtiro* flow-through diffusion cell model.

MATERIALS AND METHODS

A. Test Guidelines

The study design complies with the following test guidelines:

- OECD Guideline for the Testing of Chemicals. Draft New Guideline 428: Skin Absorption: *in vitro* Method (2002).
- OECD Draft Guidance Document for the Conduct of Skin Absorption Studies. OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28 (2002).

The metabolic activity of the skin was not confirmed prior to dosing. This exception did not affect the objectives or the validity of the study because all skin specimens were maintained with a physiological-based receptor fluid containing antibiotic from the time of harvest through the 24-hour collection, and dosing of the skin specimens occurred in less than four hours from the time of removal from donors.

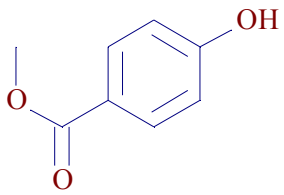
B. Test Substances

1. Methylparaben (CASN 99-76-3)

The test substance was supplied by Protameen Chemicals, Inc., assigned Haskell Laboratory Number 26201 upon receipt, and stored at room temperature.

Molecular Weight:	152
Empirical Formula:	$C_8H_8O_3$
Lot No.	M3146
Purity:	99.9%
Octanol-water partition coefficient (log P):	1.87

Structure:

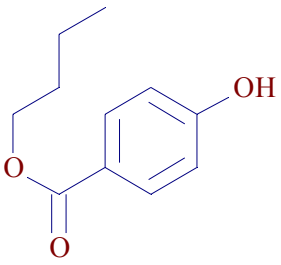


2. Butylparaben (CASN 94-26-8)

The test substance was supplied by Protameen Chemicals, Inc., assigned Haskell Laboratory Number 26202 upon receipt, and stored at room temperature.

Molecular Weight:	194
Empirical Formula:	$C_{11}H_{14}O_3$
Lot No.	B3140
Purity:	99.5%
Octanol-water partition coefficient (log P):	3.46

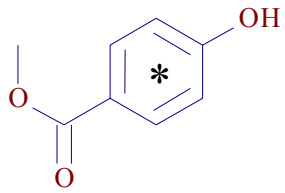
Structure:



3. Radiolabeled Test Substances

The radiolabeled test substances were supplied by Amersham Biosciences U.K. Limited, and assigned Haskell Laboratory Numbers 22705-84 (methylparaben) and 22705-85 (butylparaben) upon receipt.

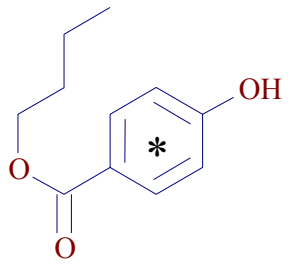
[phenyl-^{14}C(U)methylparaben



*Phenyl ring uniformly (U) labeled

Code:	CFQ13765
Batch:	1
Specific Activity:	97 µCi/mg
Purity:	99.5%

[phenyl-^{14}C(U)butylparaben



*Phenyl ring uniformly (U) labeled

Code:	CFQ13766
Batch:	1
Specific Activity:	75 µCi/mg
Purity:	99.2%

4. Formulation Vehicles

The formulation vehicles required to prepare the oil-in-water emulsions were supplied by Cosmetech Laboratories, Inc., Fairfield, New Jersey, and were assigned Haskell Laboratory Numbers 26324 (Phase A), 26325 (Phase B), and 26326 (Phase C) upon receipt. The vehicles were stored at room temperature.

C. Test System

1. Justification for Selection of Test System

Dermal contact is a route of human exposure.

The rat was the species of choice for use in the *in vitro* test system as this species has been used in toxicological evaluations of methylparaben and butylparaben. Human skin was the comparative species of choice in order to aid in the extrapolation of *in vitro* data to the human *in vivo* situation.

In vitro dermal techniques employing diffusion cell systems have been shown to predict percutaneous absorption of various chemicals *in vivo*.[4-6]

2. Rat Skin

Male Wistar rats, approximately 6-8 weeks of age, were supplied by Charles River Laboratories, Inc., Raleigh, North Carolina. Following a required quarantine period, rats were removed from stock and uniquely identified by tail markings. Rats were sacrificed by carbon dioxide

asphyxiation, the fur from the dorsal region carefully shaved using clippers, and the skin excised. Skin specimens were identified using the Haskell animal number.

3. Human Skin

Samples of human abdominal skin from local surgeons were obtained fresh. The source and identity of the skin sample (sex, anatomical locale, and approximate age of donor) was documented in the study records. Skin specimens were identified using a unique code (e.g., HCFT-26A = Human, Caucasian, Female, Thigh sample 26-A).

At the time of harvest, both rat and human skin specimens were placed in Hepes-buffered Hanks' balanced salt solution (containing 0.05 mg/L gentimicin) and were maintained on wet ice until prepared for use. All skin specimens were used in less than 4 hours following removal from donors.

D. Dose Information

1. Dose Formulation

The oil-in-water emulsions were prepared by heating Phase A and B to approximately 75°C. In separate containers for each of the paraben ester emulsions, radiolabeled and non-radiolabeled paraben was added to Phase A with mixing. Phase B was then added to Phase A, containing the paraben active ingredient, followed by the addition of Phase C. The emulsions were thoroughly mixed, cooled, and stored refrigerated at 0-10°C. A summary of the target parameters is presented in the table below.

Formulation	Target Concentration
0.8% (w/v) methylparaben emulsion	8 g methylparaben/L
0.4% (w/v) butylparaben emulsion	4 g butylparaben /L

2. Homogeneity, Concentration, and Stability

The homogeneity and amount of radiolabeled methylparaben and butylparaben (μCi/g) in each formulation was determined by subjecting aliquots of the prepared formulation to radioanalysis by liquid scintillation counting (LSC).

The concentration of methylparaben and butylparaben in each formulation was determined chromatographically using the following analytical equipment and method:

System: Agilent 1100 Series Equipment (Agilent Technologies, Palo Alto, CA, USA)
Column: Zorbax SB-C18 4.6 mm x 75 mm, 3.5 µm particles
Column temperature: Ambient
Mobile phases: A: 0.5% trifluoroacetic acid in water
B: Acetonitrile

Gradient:

Time (min)	%A	%B
0.00	90	10
6.00	0	100
7.00	0	100
7.01	90	10

Flow rate: 1 mL/min
UV Wavelength 258 nm

The results of the homogeneity and concentration analyses were used to calculate the specific activity of radiolabeled methylparaben and butylparaben (µCi/mg) in each formulation.

The purity of the neat radiolabeled paraben esters and their stability in the prepared emulsions was determined using the following analytical equipment and method:

System: Agilent 1100 Series Equipment (Agilent Technologies, Palo Alto, CA, USA)
Column: Zorbax SB-C18 4.6 mm x 150 mm, 3.5 µm particles
Column temperature: Ambient
Mobile phases: A: 2 mM ammonium acetate in water
B: Acetonitrile

Gradient:

Time (min)	%A	%B
0.00	90	10
27.00	40	60
30.00	0	100
30.01	90	10

Flow rate: 1 mL/min
Radiodetection:
- Radiomatic™ Series 500TR Flow Scintillation System, 31.9 µL CaF2 solid cell (neat radiolabeled materials)
- Fraction collection (Foxy 200™, Isco, Inc., Lincoln, NE) followed by liquid scintillation counting (prepared emulsions)

3. Dose Groups

This study was composed of the following dose groups and target parameters.

a. 0.8% (w/v) emulsion of methylparaben (8 g methylparaben/L)

Species	Group	Dose Concentration (g methylparaben/L)	Skin Dose Level (µg methylparaben/cm^2)	Number of Skin Preparations	µCi/skin
Rat	A	8	80	10	1.0
Human	B	8	80	10	1.0

b. 0.4% (w/v) emulsion of butylparaben (4 g butylparaben/L)

Species	Group	Dose Concentration (g butylparaben/L)	Skin Dose Level (µg butylparaben/cm^2)	Number of Skin Preparations	µCi/skin
Rat	C	4	40	10	1.0
Human	D	4	40	10	1.0

E. Preparation of Skin Membranes

Samples of rat and human skin were dermatomed to approximately 450 µm using a Padgett Electro Dermatome® (Padgett Instruments, Inc., Kansas City, MO).

F. *In Vitro* Diffusion Cells

An automated flow-through diffusion cell system (PermeGear, Inc., Bethlehem, PA) with an exposure area of 0.64 cm^2 and a receptor chamber volume of approximately 250 µL was used for this study. The dermatomed skin membrane was placed, *stratum corneum* uppermost, on to the top of the receptor chamber. The donor (top) chamber was then placed over the skin section and clamped in-place. A re-circulating water bath was used to maintain a receptor fluid temperature of approximately 32°C.

G. *In Vitro* Percutaneous Absorption and Metabolism of Methylparaben and Butylparaben

1. Pre-Dose Procedures

The integrity of each membrane was assessed by measurement of electrical impedance following equilibration and prior to application of test formulation.[7-8]

With the skin membranes mounted in the diffusion cells, the underside of each skin replicate was perfused with 0.9% saline at approximately 1.5 mL/h. Following a brief equilibration, an electrical impedance measurement was taken using a model 6401 Tinsley Databridge (H. Tinsley Inc., Croyden, Surry, UK). Membranes with an impedance of $\geq$4.5 k-ohms (rat) and $\geq$10.4 k-ohms (human) were considered intact and retained for use on study. Membranes not meeting the minimum impedance criteria were replaced with additional replicates, and impedance confirmed following equilibration. This procedure was followed until a minimum of 10 skin preparations represented by at least 3 individuals per species per formulation was achieved.

Following membrane selection, the saline was flushed from the flow-through system, replaced with 25 mM Hepes-buffered Hanks' balanced salt solution containing 0.05 mg/mL gentamicin and 3.75% bovine serum albumin, and equilibrated for approximately 30 minutes prior to application of the emulsion.

2. Application of the Formulated Test Substance

The prepared emulsions were applied to the skin surface, via the donor chamber, as a single finite dose at a rate of 10 µL/cm^2. The dose (6.4 µL) was distributed evenly over the skin surface using a spreader device.

The donor chamber remained unoccluded for the duration of the exposure period.

3. Dose Determination

The actual amount of radioactivity administered to the skin was measured by counting replicate mock doses and using the mean value as the amount of radioactivity applied. The amount of paraben ester applied was based on the total radioactivity determination and the verified specific activity of the formulated emulsion.

4. Exposure Period

The exposure period was 24 hours for all skin preparations.

5. Serial Sampling of Receptor Fluid

Following dose application, samples of receptor fluid were collected at 1, 2, 3, 4, 5, 6 hours, and every other hour until 24 hours.

H. Terminal Procedures

All skin preparations were washed at the conclusion of the 24-hour exposure period using at least 3 x 0.8 mL of a 2% soap solution (e.g., Ivory® Soap) followed by at least 1 x 0.8 mL rinse with DI water. The wash was collected into a liquid scintillation vial.

The donor chamber was removed and rinsed with approximately 2 mL of acetonitrile directly into a liquid scintillation vial.

The skin membrane was removed from the receptor chamber and tape-stripped 5 times using Leukotape® P (Beiersdorf, Hamburg, Germany). The tapes were placed into a glass vial and extracted with acetonitrile. The remaining skin piece was placed into a glass scintillation vial and digested using Soluene®-350.

I. Determination of Radioactivity

Liquid scintillation cocktail (e.g., Ultima Gold™ XR) was added directly to vials containing aliquots of serial receptor fluid samples, and to the entire vial contents of the skin wash, donor chamber rinse, and tape strip extracts.

Hionic-Fluor™ was added to vials containing the digested skin piece.

The samples were analyzed by liquid scintillation counting (LSC) for total radioactivity.

J. Liquid Scintillation Counting

Samples were analyzed in a Packard 2500TR or 2700TR liquid scintillation counter (Perkin-Elmer, Inc., Wellesley, Massachusetts, USA). Samples were counted for 10 minutes or until 160,000 disintegrations were accumulated (0.5%, 2σ), whichever came first.

The limit of detection (LOD) for the analysis of each sample was taken as twice the background disintegration rate obtained from analysis of appropriate blank samples.

K. Analysis of Receptor Fluid Samples

Samples of receptor fluid, along with reference standards, were mixed with acetonitrile, filtered, and analyzed for methylparaben, butylparaben, and 4-hydroxybenzoic acid using a Waters Alliance® 2795 Liquid Chromatograph (LC) coupled to a Micromass Quattro micro™ tandem quadrupole mass spectrometer (MS) equipped with a MASSLYNX data acquisition system (Micromass Inc., Manchester, UK).

HPLC Conditions:

Column: Agilent Zorbax SB-C18, 2.1 x 30 mm, 3.5 µm particles
Column temperature: Ambient
Injection volume: 5 µL
Solvent: A: 0.5% formic acid in water
B: Acetonitrile

Gradient:

Time (min)	A	B
0	97	3
4.00	0	100
7.00	0	100
7.01	97	3

Flow rate: 0.30 mL/min
Run time:: 10 minutes

MS Conditions:

Ionization mode:	Electrospray negative (ES-)
Capillary voltage:	3.20 kV
Cone voltage	(a) 21 V for 4HBA
	(b) 29 V for MP
	(c) 31 V for BP
Extractor voltage:	2.0 V
Source temperature:	140°C
Cone temperature:	Not controlled
Desolvation temperature:	350°C
Cone gas flow (approx.):	100 L/Hr (nitrogen)
Desolvation gas flow (approx.):	650 L/Hr (nitrogen)
Collision energy:	(a) 13 eV for 4HBA
	(b) 18 eV for MP
	(c) 22 eV for BP
Collision gas::	Argon
Mode:	Multiple Reaction Monitoring (MRM)
	(a) 151.08 > 91.78 for MP
	(b) 153.08 > 93.78 for ^{14}C-MP
	(c) 137.03 > 92.78 for 4HBA
	(d) 139.03 > 94.78 for ^{14}C-4HBA
	(e) 193.20 > 91.90 for BP
	(f) 195.20 > 93.90 for ^{14}C-BP

From each formulation and species, a representative collection of serial receptor fluid samples from a single skin were pooled and analyzed using the radiochemical purity method with fraction collection. The individual fractions were quantitated by liquid scintillation counting and a reconstructed radiochromatogram generated. Areas of interest were submitted for identification by mass spectrometry. Mass spectral analysis of the isolated fractions was performed using the following equipment and methods:

LC/MS system: Waters Alliance® 2790 Liquid Chromatograph (LC) coupled to a Micromass QtoF II™ hybrid quadrupole/ time-of-flight mass spectrometer (MS) equipped with a MASSLYNX data acquisition system (Micromass Inc., Manchester, UK)

HPLC Conditions

Column: Agilent Sorbax SB-C_{18} RP, 2.1 x 150 mm, 3.5 µm particle size

Column temperature: Ambient

Solvent: A: 0.15% acetic acid in water
B: Methanol

Gradient:

Time (min)	A	B
0.00	90	10
2.00	90	10
35.00	0	100
45.00	0	100
45.10	90	10
50.00	90	10

Flow rate: 0.30 mL/min

MS Conditions

Ionization mode: Electrospray negative (ES-)

Capillary voltage: -2.85 kV

Cone voltage 16 V

Extractor voltage: 0 V

Source temperature: 125°C

Desolvation temperature: 350°C

Cone gas flow: 60 L/Hr (nitrogen)

Desolvation gas flow: 500 L/Hr (nitrogen)

Collision energy: 4.6 eV (MS only mode); 25 eV (MS/MS mode)

Mode: Full Scan MS only for metabolite screening
Full Scan MS/MS for metabolite identification

L. Semi-Quantitative Analysis of Prepared Emulsions for the Presence of Ethanol

Approximately 100 mg of the each formulation was diluted in 2 mL of HPLC grade water and extracted with 1 mL dichloromethane (methylene chloride). The dichloromethane layer was transferred to a suitable vial and submitted for analysis by gas chromatography/mass spectrometry (GC/MS), along with a process control sample (blank), and an ethanol standard. The reconstructed single ion chromatogram of the molecular ion of ethanol (*m/z* 46) was used to estimate the relative amounts of ethanol in the formulations using a single point calibration method. The GC/MS equipment and operating parameters are given below.

System:	Agilent Gas Chromatography (GC) HP 6890 Series/Mass Selective Detection (MSD) HP 5973 (Agilent Technologies, Palo Alto, CA, USA)	
Column:	DB-1701 Capillary Column, 30 m x 0.320 mm x 1.0 µm (J&W Scientific, Folsom, CA USA)	
Inlet Program:	Initial Temperature:	250°C
	Pressure:	12.45 psi
	Split Ratio:	10:1
	Split Flow:	37.0 mL/min
	Total Flow:	43.7 mL/min
Temperature:	Initial:	60°C
	Final:	225°C
	Hold Time:	1 minute; ramp 7.5°C/min
MS Detector:	Acquisition Mode:	Full Scan
	Solvent Delay:	0.50 min
	Ions/Dwell in Group:	
	Mass:	28/350
	Dwell:	0.95 sec
	MS Quad:	150°C
	MS Source:	230°C

M. Statistical Analyses and Data Presentation

Group data is represented as Mean ± SD. The statistical significance of selected data was assessed by the Student's T-test using Microcal™, version 7.0 (OriginLab Corporation, Northhampton, Massachusetts). A p-value of ≤0.05 was considered significant.

RESULTS AND DISCUSSION

A. Purity of Radiolabeled Methylparaben and Butylparaben (Figures 1-2, Appendix A)

The radiochemical purity of the neat [^{14}C]-methylparaben and [^{14}C]-butylparaben (Amersham Biosciences UK Limited) was determined to be greater than 99%. Representative radiochromatograms are presented in Figures 1 and 2.

B. Storage Stability of [^{14}C]-Active Ingredients in Emulsions (Figures 3-4, Appendix A)

When incorporated into the oil-in-water emulsions, and stored refrigerated 0-10°C, both radiolabeled active ingredients were stable (>99%) for approximately 3 months (Figures 3 and 4). Therefore, the formulated emulsions were stable under the conditions used in this study.

Documents relating to the formulation ingredients and procedures for preparation of the emulsions from Cosmetech Inc., along with certificates of analysis (COA) for non-radiolabeled methylparaben and butylparaben, and 4-hydroxybenzoic acid reference material, are presented in Appendix A.

C. Verified Concentrations for the Emulsions

The HPLC-UV verified chemical concentration for the 0.8% methylparaben emulsion (0.75%) and the 0.4% butylparaben emulsion (0.41%) was comparable to the target concentrations.

D. 0.8% Methylparaben, Rat (Tables 1-5, Figures 5-9, Appendix B)

Data for total radioactivity applied (µCi), the total amount of methylparaben applied (µg), and the application rate (µg/cm²) for methylparaben is presented in Table 1.

Key observations of mean data:

- The cumulative amount of radioactivity, methylparaben, and 4-hydroxybenzoic acid absorbed per area at 24 hours was 34.85 µg equiv/cm^2, 8.30 µg/ cm^2, and 18.83 µg/ cm^2, respectively (Table 2, Figure 5).

- Based on the concentration-time course data, the peak for total radioactivity (2.46 µg equiv/mL), methylparaben (0.59 µg/mL), and 4-hydroxybenzoic acid (1.75 µg/mL) occurred at 2 hours, post-application (Table 3, Figure 6).

- The cumulative amount absorbed at 24 hours for total radioactivity, methylparaben, and 4-hydroxybenzoic acid was 54.94%, 13.10%, and 29.64%, respectively (Table 4, Figure 7); of the total radioactivity absorbed, methylparaben and 4-hydroxybenzoic acid represented 23.8% and 53.9%, respectively.

- At the end of the exposure phase, the total amount of radioactivity absorbed (receptor fluid plus receptor wash), the absorbable fraction (absorbed plus tape-stripped skin), and the unabsorbed dose (wash, donor chamber rinse, tape strips) was 55.37%, 67.61% and 23.49%, respectively (Table 5, Figures 8-9); 91.09% of the applied radioactivity was recovered at the end of the 24-hour exposure phase.

These data show that following application of a 0.8% methylparaben emulsion to viable rat skin that of the total radioactivity detected in the receptor fluid (54.94%), a major portion (53.9%) had been metabolized to 4-hydroxybenzoic acid, with a smaller portion (23.8%) accounted for as unmetabolized methylparaben.

E. 0.8% Methylparaben, Human (Tables 6-10, Figures 10-14, Appendix C)

Data for radioactivity applied (µCi), the total amount of methylparaben applied (µg), and the application rate (µg/cm²) for methylparaben is presented in Table 6. These amounts and rates were comparable to those for rat skin (Table 1).

Key observations of mean data:

- The cumulative amount of radioactivity, methylparaben, and 4-hydroxybenzoic acid absorbed per area at 24 hours was 48.86 µg equiv/cm^2, 29.56 µg/ cm^2, and 17.11 µg/ cm^2, respectively (Table 7, Figure 10).

- Based on the concentration-time course data, the peak for total radioactivity (3.42 µg equiv/mL), methylparaben (2.96 µg/mL), and 4-hydroxybenzoic acid (1.00 µg/mL) occurred at 2 hours, post-application (Table 8, Figure 11).

- The cumulative amount absorbed at 24 hours for total radioactivity, methylparaben, and 4-hydroxybenzoic acid was 79.36%, 47.84%, and 27.83%, respectively (Table 9, Figure 12); of the total radioactivity absorbed, methylparaben and 4-hydroxybenzoic acid represented 60.3% and 35.1%, respectively.

- At the end of the exposure phase, the total amount of radioactivity absorbed (receptor fluid plus receptor wash), the absorbable fraction (absorbed plus tape-stripped skin), and the unabsorbed dose (wash, donor chamber rinse, tape strips) was 79.82%, 84.69% and 21.21%, respectively (Table 10, Figures 13-14); 105.91% of the applied radioactivity was recovered at the end of the 24-hour exposure phase.

These data show that following application of a 0.8% methylparaben emulsion to viable human skin that of the total radioactivity detected in the receptor fluid (79.36%), a portion (35.1%) had been metabolized to 4-hydroxybenzoic acid, with the majority (60.3%) accounted for as unmetabolized methylparaben.

F. 0.4% Butylparaben, Rat (Tables 11-15, Figures 15-19, Appendix D)

Data for the total radioactivity applied (µCi), the total amount of butylparaben applied (µg), and the application rate (µg/cm²) for butylparaben are presented in Table 11.

Key observations of mean data:

- The cumulative amount of radioactivity, butylparaben, and 4-hydroxybenzoic acid absorbed per area at 24 hours was 19.53 µg equiv/cm^2, 1.08 µg/ cm^2, and 10.18 µg/ cm^2, respectively (Table 12, Figure 15).

- Based on the concentration-time course data, the peak for total radioactivity (0.83 μg equiv/mL), butylparaben (0.05 μg/mL), and 4-hydroxybenzoic acid (0.44 μg/mL) occurred at 2-3 hours, post-application (Table 13, Figure 16).

- The cumulative amount absorbed at 24 hours for total radioactivity, butylparaben, and 4-hydroxybenzoic acid was 54.23%, 2.99%, and 28.38%, respectively (Table 14, Figure 17); of the total radioactivity absorbed, butylparaben and 4-hydroxybenzoic acid represented 5.51% and 52.3%, respectively.

- At the end of the exposure phase, the total amount of radioactivity absorbed (receptor fluid plus receptor wash), the absorbable fraction (absorbed plus tape-stripped skin), and the unabsorbed dose (wash, donor chamber rinse, tape strips) was 54.67%, 67.69% and 30.99%, respectively (Table 15, Figures 18-19); 98.68% of the applied radioactivity was recovered at the end of the 24-hour exposure phase.

These data show that following application of a 0.4% butylparaben emulsion to viable rat skin that of the total radioactivity detected in the receptor fluid (54.23%), a major portion (52.3%) had been metabolized to 4-hydroxybenzoic acid, with a minor portion (5.51%) accounted for as unmetabolized butylparaben.

G. 0.4% Butylparaben, Human (Tables 16-20, Figures 20-24, Appendix E)

Data for the total radioactivity applied (μCi), the total amount of butylparaben applied (μg), and the application rate (μg/cm²) for butylparaben are presented in Table 16. These amounts and rates were comparable to those for rat skin (Table 11).

Key observations of mean data:

- The cumulative amount of radioactivity, butylparaben, and 4-hydroxybenzoic acid absorbed per area at 24 hours was 26.03 μg equiv/cm^2, 12.99 μg/ cm^2, and 8.52 μg/ cm^2, respectively (Table 17, Figure 20).

- Based on the concentration-time course data, the peak for total radioactivity (1.09 μg equiv/mL), butylparaben (0.77 μg/mL), and 4-hydroxybenzoic acid (0.26 μg/mL) occurred at 3-4 hours, post-application (Table 18, Figure 21).

- The cumulative amount absorbed at 24 hours for total radioactivity, butylparaben, and 4-hydroxybenzoic acid was 73.51%, 36.57%, and 24.08%, respectively (Table 19, Figure 22); of the total radioactivity absorbed, butylparaben and 4-hydroxybenzoic acid represented 49.7% and 32.8%, respectively.

- At the end of the exposure phase, the total amount of radioactivity absorbed (receptor fluid plus receptor wash), the absorbable fraction (absorbed plus tape-stripped skin), and the unabsorbed dose (wash, donor chamber rinse, tape strips) was 74.23%, 81.15% and 17.66%,

respectively (Table 20, Figures 23-24); 98.81% of the applied radioactivity was recovered at the end of the 24-hour exposure phase.

These data show that following application of a 0.4% butylparaben emulsion to viable human skin that of the total radioactivity detected in the receptor fluid (73.51%), a smaller portion (32.8%) had been metabolized to 4-hydroxybenzoic acid, with a major portion (49.7%) accounted for as unmetabolized butylparaben.

H. HPLC-Radiochromatograhic Analysis of Receptor Fluid Pools (Figures 25-28)

1. Methylparaben Receptor Fluid Pool

HPLC-radiochromatograms of pooled receptor fluid samples from the methylparaben experiment are presented in Figures 25-26. Exclusive of species, three peaks were observed with approximate retentions times of 2.5, 12.5 and 17.5 minutes.

2. Butylparaben Receptor Fluid Pool

HPLC-radiochromatograms of pooled receptor fluid samples from the butylparaben experiment are presented in Figures 27-28. Three peaks were observed in the representative human pool with approximate retentions times of 2.5, 17.5 and 24.5 minutes. While in the rat receptor fluid pooled sample, only peaks at 2.5 and 17.5 minutes were noted.

I. Mass Spectrometral Analysis of Isolated Fractions from Receptor Fluid Pools (Figures 29-36)

1. Methylparaben Fractions

The negative electrospray mass spectrum and daughter ion mass spectrum of unknown at 2.5 minutes (Figures 29 and 30) was consistent with the 4-hydroxybenzoic acid standard. Likewise, the negative electrospray mass spectrum and daughter ion mass spectrum of unknown at 12.5 minutes (Figures 31 and 32) was consistent with methylparaben and was identical to the standard. These ions are diagnostic of the relative acidities of the molecular ion species formed in negative ion electrospray. The hydroxybenzoic acid donates its carboxylic proton most readily while the only available acidic proton in the ester is the phenolic proton. Once in the anionic form, both molecules fragment in the collision cell via decarboxylation to give rise to the characteristically intense 93 Da (acid) and 92 Da (ester) ions. The negative electrospray mass spectrum and daughter ion mass spectrum of unknown at 17.5 minutes (Figures 33 and 34) was consistent with ethylparaben.

2. Butylparaben Fractions

The negative electrospray mass spectrum and daughter ion mass spectrum of unknowns at 2.5 and 17.5 minutes were consistent with the 4-hydroxybenzoic acid and ethylparaben, respectively.

The negative electrospray mass spectrum and daughter ion mass spectrum of unknown at 24.5 minutes (Figures 35 and 36) was consistent with butylparaben and are identical to the standard.

J. Semi-Quantitative Analysis of Prepared Emulsions for the Presence of Ethanol (Figures 37-40)

Based on the GC/MS results, both the methylparaben and butylparaben emulsion formulations were found to contain ethanol. The concentration of ethanol in the formulations, based on the assumption of 100% partition of ethanol from the emulsions into the dichloromethane, was estimated to be 0.074 mg/mL and 0.055 mg/mL for the methylparaben and butylparaben formulations, respectively. The source of the ethanol in the formulations was not determined.

K. Biotransformation Route, Formation of Ethylparaben (Figure 41)

The primary biotransformation route for both methylparaben and butylparaben in this dermal penetration-metabolism study using a dermatomed human skin model was hydrolysis of the paraben ester to the more water-soluble 4-hydroxybenzoic acid (para-hydroxybenzoic acid).

The dermatomed viable skin model used in the current study contained an intact *stratum corneum*, epidermis, and a small portion of the upper dermis region with the underlying hypodermis and subcutaneous fat removed. However, previous experiments using human skin have demonstrated that the most prominent esterase from subcutaneous fat preferred methylparaben, while esterase extracts from keratinocytes in the *stratum corneum* and viable epidermis preferred the longer chain butylparaben.[9] The specific activities of the esterases in subcutaneous fat and keratinocytes may explain the results reported here.

Exclusive of species, the concentration of unmetabolized methylparaben in receptor fluid was greater than the concentration of unmetabolized butylparaben likely due to the dermatomed viable skin model with an intact *stratum corneum* (containing esterases with a preference toward ester hydrolysis of butylparaben) and the absence of subcutaneous fat (containing esterases with a preference toward ester hydrolysis of methylparaben). Alternatively, the higher amount of unmetabolized methylparaben in the receptor fluid could be due to its physicochemical properties (lower MW and LogP compared to butylparaben) resulting in a longer residence time for butylparaben (higher MW, greater lipohilicity) which was metabolized to a greater extent.

Biotransformation of either paraben ester to ethylparaben was not expected. As a result, quantitative analysis of receptor fluid samples for ethylparaben was not performed. Therefore, it was not established whether the differences between total radioactivity and the parent plus 4-hydroxybenzoic acid was due to ethylparaben. Nonetheless, no other compounds (metabolites) were detected in the receptor fluid samples. However, one plausible explanation might be the presence of ethanol in the test formulation which resulted in the formation of ethylparaben. The presence of ethanol could form ethylparaben via transesterification with the parent chemical or esterification with 4-hydroxybenzoic acid as shown in Figure 41.[10] Formation of ethylparaben and 4-hydroxybenzoic acid in excised skin from a Yucatan micropig, which possesses

similarities to human skin, can only occur in the presence of methylparaben, ethanol, and most importantly, skin homogenates (suggesting an enzymatic process).[1] The fact that ethylparaben and 4-hydroxybenzoic acid were not seen in the starting emulsions, but were detected in the receptor fluid pools as products of metabolism, is consistent with the presence of ethanol.

CONCLUSIONS

A. Methylparaben, 0.8% Oil-in-Water Emulsion

Following application of a 0.8% methylparaben emulsion to viable rat and human skin, a greater amount of total radioactivity penetrated human skin (79.36%) compared to rat skin (54.94%). A major portion of the total radioactivity that had penetrated rat skin was metabolized to 4-hydroxybenzoic acid (53.9%), with a smaller portion (23.8%) accounted for as unmetabolized methylparaben. By comparison, a lesser portion of the total radioactivity that had penetrated viable human skin had been metabolized to 4-hydroxybenzoic acid (35.1%), with the majority (60.3%) accounted for as unmetabolized methylparaben. Exclusive of species, analysis of receptor fluid pools by radiochromatography and LC-MS revealed the presence of methylparaben, 4-hydroxybenzoic acid, and ethylparaben.

B. Butylparaben, 0.4% Oil-in-Water Emulsion

Following application of a 0.4% butylparaben emulsion to viable rat and human skin, a greater amount of total radioactivity penetrated human skin (73.51%) compared to rat skin (54.23%). A majority of the total radioactivity that had penetrated rat skin was metabolized to the primary acid metabolite 4-hydroxybenzoic acid (52.3%), with a smaller portion (5.51%) accounted for as unmetabolized butylparaben. For human skin, 32.8% of the total radioactivity in the receptor fluid had been metabolized to 4-hydroxybenzoic acid, with a greater portion (49.7%) accounted for as unmetabolized butylparaben. As was observed for the methylparaben formulation, analysis of receptor fluid pools by radiochromatography and LC-MS revealed the presence of butylparaben, 4-hydroxybenzoic acid, and ethylparaben.

Overall these data show, based on a dermatomed viable rat and human skin model and analysis of receptor fluid samples, that the dermal bioavailability of methylparaben and butylparaben from the oil-in-water emulsions was greater for human skin than rat skin, and that a small portion of the total radioactivity that had penetrated the skin, exclusive of species and formulation, was ethylparaben.

RECORDS AND SAMPLE STORAGE

All data and records for analytical characterizations conducted by the sponsor will be retained by the sponsor. Raw data and the final report will be retained at Haskell Laboratory, Newark,

Delaware, or at Iron Mountain Records Management, Wilmington, Delaware, and will be returned to the sponsor within 6 months after the final report issues.

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TABLES

TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

absorbed	receptor fluid + receptor wash
absorbable	absorbed + skin
equiv	equivalent
SD	standard deviation
unabsorbed	skin wash + donor chamber + tape strips

Table 1: Methylparaben – rat – application amounts and rates

	Mean	SD
Activity applied (µCi)	0.80	0.01
Total Methylparaben (µg)	40.61	0.29
Methylparaben per area (µg/cm^2)	63.45	0.45

Table 2: Methylparaben – rat – cumulative amount absorbed per area

Time (hours)	Radioactivity (µg equiv/cm^2)		Methylparaben (µg/cm^2)		4-hyroxybenzoic acid (µg/cm^2)	
	Mean	SD	Mean	SD	Mean	SD
1	2.97	2.51	0.77[a]	0.73	0.94	1.24
2	9.04	5.26	2.24[a]	1.74	5.26	4.51
3	12.71	6.20	3.27[a]	2.91	7.31	5.22
4	14.93[a]	6.41	3.72[a]	3.25	8.52	5.45
5	16.58[a]	6.37	3.93[a]	3.31	9.10	5.69
6	17.91[a]	6.28	4.21[a]	3.41	9.79	5.75
8	20.40[a]	5.93	4.77[a]	3.55	11.09	5.84
10	22.61[a]	5.93	5.31[a]	3.68	12.35	5.85
12	24.73[a]	4.99	5.72[a]	3.76	13.16	6.00
14	26.79[a]	4.56	6.29[a]	3.93	14.36	6.05
16	28.68[a]	4.20	6.81[a]	4.10	15.46	6.13
18	30.43[a]	3.94	7.28[a]	4.25	16.51	6.24
20	32.04[a]	3.77	7.56[a]	4.35	17.11	6.36
22	33.53[a]	3.68	7.96[a]	4.49	18.02	6.49
24[b]	34.85[a]	3.71	8.30[a]	4.62	18.83	6.63

[a] Statistically different from methylparaben human ($p < 0.05$).
[b] Cumulative amount absorbed at 24 hours does not include receptor wash.

Table 3: Methylparaben – rat – concentration-time course

Time (hours)	Radioactivity (µg equiv/mL)		Methylparaben (µg/mL)		4-hyroxybenzoic acid (µg/mL)	
	Mean	SD	Mean	SD	Mean	SD
1	1.20	1.02	0.31	0.29	0.38	0.50
2	2.46	1.17	0.59	0.50	1.75	1.55
3	1.49	0.56	0.42	0.52	0.83	0.39
4	0.90	0.30	0.18	0.15	0.49	0.24
5	0.67	0.21	0.08	0.05	0.23	0.17
6	0.54	0.15	0.12	0.07	0.28	0.14
8	0.50	0.14	0.11	0.07	0.26	0.13
10	0.45	0.15	0.11	0.07	0.25	0.13
12	0.43	0.15	0.08	0.06	0.16	0.08
14	0.42	0.16	0.12	0.07	0.24	0.12
16	0.38	0.15	0.11	0.06	0.22	0.11
18	0.35	0.14	0.09	0.05	0.21	0.10
20	0.32	0.13	0.06	0.03	0.12	0.06
22	0.30	0.13	0.08	0.04	0.18	0.10
24	0.27	0.11	0.07	0.04	0.16	0.09

Table 4: Methylparaben – rat – cumulative percent absorbed

Time	Radioactivity		Methylparaben		4-hyroxybenzoic acid	
(hours)	Mean	SD	Mean	SD	Mean	SD
1	4.68	3.95	1.22[a]	1.14	1.48	1.95
2	14.24	8.29	3.53[a]	2.75	8.28	7.07
3	20.02[a]	9.74	5.16[a]	4.58	11.50	8.18
4	23.51[a]	10.05	5.86[a]	5.10	13.40	8.53
5	26.11[a]	9.98	6.19[a]	5.19	14.30	8.90
6	28.21[a]	9.82	6.64[a]	5.35	15.39	9.00
8	32.12[a]	9.25	7.52[a]	5.58	17.44	9.12
10	35.61[a]	8.50	8.38[a]	5.79	19.43	9.13
12	38.95[a]	7.77	9.02[a]	5.92	20.71	9.36
14	42.20[a]	7.10	9.92[a]	6.18	22.60	9.44
16	45.19[a]	6.56	10.75[a]	6.46	24.34	9.56
18	47.95[a]	6.18	11.49[a]	6.71	25.98	9.72
20	50.49[a]	5.94	11.92[a]	6.86	26.93	9.91
22	52.84[a]	5.84	12.55[a]	7.08	28.36	10.12
24[b]	54.94[a]	5.92	13.10[a]	7.30	29.64	10.35

[a] Statistically different from methylparaben human ($p < 0.05$).
[b] Cumulative amount absorbed at 24 hours does not include receptor wash.

Table 5: Methylparaben – rat – material balance

		Mean	SD
Absorbed dose			
	Receptor fluid	54.94	5.92
	Receptor wash	0.43	0.20
	Total absorbed	55.37	5.92
Absorbable dose			
	Receptor fluid	54.94	5.92
	Receptor wash	0.43	0.20
	Skin	12.23	5.57
	Total absorbable	67.61	6.06
Unabsorbed dose			
	Skin wash	17.81	2.82
	Donor chamber	0.03	0.01
	Tape strips	5.65	1.12
	Total unabsorbed	23.49	2.40
Total recovered		91.09	5.66

Table 6: Methylparaben – human – application amounts and rates

	Mean	SD
Activity applied (μCi)	0.78	0.02
Total Methylparaben (μg)	39.41	1.09
Methylparaben per area (μg/cm^2)	61.58	1.70

Table 7: Methylparaben – human – cumulative amount absorbed per area

Time (hours)	Radioactivity (µg equiv/cm^2)		Methylparaben (µg/cm^2)		4-hyroxybenzoic acid (µg/cm^2)	
	Mean	SD	Mean	SD	Mean	SD
1	2.40	1.44	2.22[a]	1.89	0.32	0.29
2	10.86	2.73	9.52[a]	8.77	2.79	2.70
3	16.74	2.96	13.41[a]	9.19	4.89	4.30
4	20.53[a]	3.18	15.58[a]	9.55	6.38	5.21
5	23.52[a]	3.45	16.90[a]	10.13	7.39	5.65
6	25.98[a]	3.73	18.04[a]	10.38	8.20	6.06
8	29.73[a]	4.30	19.84[a]	10.77	9.45	6.69
10	33.00[a]	5.10	21.75[a]	11.18	10.73	7.34
12	36.09[a]	5.99	23.36[a]	11.74	11.75	7.61
14	38.88[a]	6.84	24.91[a]	12.00	13.08	8.33
16	41.50[a]	7.62	26.24[a]	12.15	14.18	8.91
18	43.75[a]	8.29	27.39[a]	12.28	15.21	9.42
20	45.72[a]	8.87	28.24[a]	12.47	15.93	9.65
22	47.38[a]	9.24	28.96[a]	12.52	16.58	9.97
24[b]	48.86[a]	9.61	29.56[a]	12.60	17.11	10.19

[a] Statistically different from methylparaben rat ($p < 0.05$).
[b] Cumulative amount absorbed at 24 hours does not include receptor wash.

Table 8: Methylparaben – human – concentration-time course

Time (hours)	Radioactivity (µg equiv/mL)		Methylparaben (µg/mL)		4-hyroxybenzoic acid (µg/mL)	
	Mean	SD	Mean	SD	Mean	SD
1	0.97	0.58	0.90	0.77	0.13	0.12
2	3.42	0.69	2.96	2.94	1.00	1.00
3	2.38	0.61	1.57	0.71	0.85	0.67
4	1.54	0.50	0.88	0.44	0.60	0.39
5	1.21	0.33	0.54	0.36	0.41	0.21
6	1.00	0.27	0.46	0.19	0.33	0.18
8	0.76	0.23	0.36	0.14	0.25	0.14
10	0.66	0.21	0.39	0.20	0.26	0.15
12	0.62	0.21	0.33	0.16	0.21	0.09
14	0.56	0.24	0.31	0.11	0.27	0.18
16	0.53	0.17	0.27	0.08	0.22	0.13
18	0.45	0.15	0.23	0.07	0.21	0.11
20	0.40	0.13	0.17	0.05	0.15	0.07
22	0.33	0.12	0.15	0.04	0.13	0.07
24	0.30	0.11	0.12	0.03	0.11	0.06

Table 9: Methylparaben – human – cumulative percent absorbed

Time	Radioactivity		Methylparaben		4-hyroxybenzoic acid	
(hours)	Mean	SD	Mean	SD	Mean	SD
1	3.92	2.36	3.60[a]	3.06	0.52	0.49
2	17.70	4.73	15.46[a]	14.25	4.56	4.48
3	27.27[a]	5.33	21.73[a]	14.91	7.99	7.12
4	33.43[a]	5.73	25.22[a]	15.46	10.42	8.62
5	38.27[a]	6.14	27.36[a]	16.36	12.06	9.34
6	42.26[a]	6.55	29.21[a]	16.75	13.37	10.00
8	48.34[a]	7.35	32.11[a]	17.33	15.41	11.03
10	53.64[a]	8.56	35.20[a]	17.94	17.48	12.07
12	58.65[a]	9.91	37.81[a]	18.82	19.13	12.52
14	63.18[a]	11.22	40.30[a]	19.20	21.29	13.67
16	67.42[a]	12.44	42.46[a]	19.41	23.08	14.60
18	71.07[a]	13.49	44.32[a]	19.59	24.75	15.44
20	74.27[a]	14.42	45.69[a]	19.88	25.92	15.80
22	76.96[a]	15.02	46.86[a]	19.96	26.97	16.31
24[b]	79.36[a]	15.62	47.84[a]	20.07	27.83	16.68

[a] Statistically different from methylparaben rat ($p < 0.05$).
[b] Cumulative amount absorbed at 24 hours does not include receptor wash.

Table 10: Methylparaben – human – material balance

		Mean	SD
Absorbed dose			
	Receptor fluid	79.36	15.62
	Receptor wash	0.46	0.11
	Total absorbed	79.82	15.60
Absorbable dose			
	Receptor fluid	79.36	15.62
	Receptor wash	0.46	0.11
	Skin	4.88	2.01
	Total absorbable	84.69	15.46
Unabsorbed dose			
	Skin wash	14.65	8.76
	Donor chamber	0.42	0.94
	Tape strips	6.13	12.01
	Total unabsorbed	21.21	20.48
Total recovered		105.91	15.10

Table 11: Butylparaben – rat – application amounts and rates

	Mean	SD
Activity applied (μCi)	0.96	0.03
Total Butylparaben (μg)	23.06	0.82
Butylparaben per area (μg/cm^2)	36.03	1.27

Table 12: Butylparaben – rat – cumulative amount absorbed per area

Time (hours)	Radioactivity (µg equiv/cm^2)		Butylparaben (µg/cm^2)		4-hyroxybenzoic acid (µg/cm^2)	
	Mean	SD	Mean	SD	Mean	SD
1	0.33	0.23	0.05	0.06	0.14	0.06
2	2.36	0.93	0.17[a]	0.14	1.22[a]	0.55
3	4.41	1.65	0.30[a]	0.23	2.29[a]	0.84
4	6.08	2.26	0.38[a]	0.28	3.14[a]	1.12
5	7.35	2.43	0.44[a]	0.31	3.96[a]	1.32
6	8.50[a]	2.64	0.48[a]	0.33	4.53[a]	1.45
8	10.37[a]	2.82	0.56[a]	0.37	5.43[a]	1.63
10	12.12[a]	3.03	0.63[a]	0.40	6.21[a]	1.75
12	13.54[a]	3.15	0.73[a]	0.46	7.07[a]	1.92
14	14.87[a]	3.24	0.80[a]	0.51	7.85[a]	2.07
16	16.08[a]	3.32	0.87[a]	0.54	8.51[a]	2.20
18	17.12[a]	3.43	0.94[a]	0.58	9.06[a]	2.23
20	18.02[a]	3.46	1.01[a]	0.63	9.51	2.24
22	18.83[a]	3.56	1.05[a]	0.65	9.83	2.23
24[b]	19.53[a]	3.57	1.08[a]	0.66	10.18	2.28

[a] Statistically different from butylparaben human ($p < 0.05$).
[b] Cumulative amount absorbed at 24 hours does not include receptor wash.

Table 13: Butylparaben – rat – concentration-time course

Time (hours)	Radioactivity (µg equiv/mL)		Butylparaben (µg/mL)		4-hyroxybenzoic acid (µg/mL)	
	Mean	SD	Mean	SD	Mean	SD
1	0.14	0.09	0.02	0.02	0.06	0.03
2	0.82	0.31	0.05	0.04	0.44	0.20
3	0.83	0.36	0.05	0.04	0.43	0.17
4	0.68	0.27	0.03	0.02	0.35	0.14
5	0.51	0.13	0.02	0.02	0.33	0.11
6	0.47	0.13	0.02	0.01	0.23	0.07
8	0.38	0.11	0.02	0.01	0.18	0.06
10	0.35	0.10	0.01	0.01	0.16	0.05
12	0.29	0.07	0.02	0.02	0.17	0.06
14	0.27	0.06	0.01	0.01	0.16	0.06
16	0.24	0.07	0.01	0.01	0.13	0.05
18	0.21	0.05	0.01	0.01	0.11	0.04
20	0.18	0.04	0.01	0.01	0.09	0.03
22	0.16	0.04	0.01	0.01	0.07	0.03
24	0.14	0.03	0.01	0.01	0.07	0.02

Table 14: Butylparaben – rat – cumulative percent absorbed

Time (hours)	Radioactivity Mean	Radioactivity SD	Butylparaben Mean	Butylparaben SD	4-hyroxybenzoic acid Mean	4-hyroxybenzoic acid SD
1	0.93	0.63	0.15	0.17	0.38	0.18
2	6.53	2.51	0.46[a]	0.38	3.39[a]	1.49
3	12.18	4.38	0.82[a]	0.63	6.35[a]	2.29
4	16.83	6.01	1.04[a]	0.78	8.72[a]	3.06
5	20.35	6.48	1.19[a]	0.85	11.02[a]	3.66
6	23.55[a]	7.02	1.31[a]	0.91	12.60[a]	4.01
8	28.76[a]	7.57	1.53[a]	1.01	15.12[a]	4.61
10	33.63[a]	8.19	1.73[a]	1.11	17.29[a]	4.99
12	37.58[a]	8.51	2.00[a]	1.28	19.67[a]	5.54
14	41.27[a]	8.80	2.20[a]	1.39	21.87[a]	6.05
16	44.65[a]	9.07	2.39[a]	1.48	23.71[a]	6.51
18	47.54[a]	9.43	2.59[a]	1.61	25.26	6.69
20	50.03[a]	9.54	2.77[a]	1.73	26.52	6.78
22	52.30[a]	9.84	2.90[a]	1.79	27.42	6.80
24[b]	54.23[a]	9.89	2.99[a]	1.83	28.38	6.96

[a] Statistically different from butylparaben human ($p < 0.05$).
[b] Cumulative amount absorbed at 24 hours does not include receptor wash.

Table 15: Butylparaben – rat – material balance

		Mean	SD
Absorbed dose			
	Receptor fluid	54.23	9.89
	Receptor wash	0.44	0.10
	Total absorbed	54.67	9.88
Absorbable dose			
	Receptor fluid	54.23	9.89
	Receptor wash	0.44	0.10
	Skin	13.01	9.44
	Total absorbable	67.69	9.06
Unabsorbed dose			
	Skin wash	18.29	6.33
	Donor chamber	0.44	0.95
	Tape strips	12.27	5.45
	Total unabsorbed	30.99	7.94
Total recovered		98.68	5.64

Table 16: Butylparaben – human – application amounts and rates

	Mean	SD
Activity applied (μCi)	0.94	0.02
Total Butylparaben (μg)	22.68	0.42
Butylparaben per area (μg/cm^2)	35.43	0.66

Table 17: Butylparaben – human – cumulative amount absorbed per area

Time (hours)	Radioactivity (µg equiv/cm^2)		Butylparaben (µg/cm^2)		4-hyroxybenzoic acid (µg/cm^2)	
	Mean	SD	Mean	SD	Mean	SD
1	0.22	0.17	0.10	0.10	0.10	0.07
2	2.18	1.02	1.55[a]	1.08	0.62[a]	0.31
3	4.88	1.64	3.44[a]	2.43	1.23[a]	0.50
4	7.09	1.80	4.96[a]	3.09	1.87[a]	0.69
5	8.96	1.88	6.05[a]	3.42	2.42[a]	0.80
6	10.73[a]	1.99	6.89[a]	3.67	2.89[a]	0.89
8	13.36[a]	2.38	8.04[a]	4.02	3.70[a]	1.11
10	15.97[a]	2.60	11.12[a]	5.16	4.05[a]	1.35
12	17.92[a]	3.00	12.23[a]	5.18	4.69[a]	1.57
14	19.82[a]	3.16	13.11[a]	5.21	5.31[a]	1.73
16	21.03[a]	3.33	11.52[a]	5.19	6.54[a]	1.91
18	22.44[a]	3.41	12.00[a]	5.26	7.11[a]	2.12
20	23.71[a]	3.54	12.40[a]	5.30	7.63	2.33
22	24.95[a]	3.61	12.73[a]	5.35	8.13	2.52
24	26.03[a]	3.60	12.99[a]	5.38	8.52	2.66

[a] Statistically different from butylparaben rat ($p < 0.05$).
[b] Cumulative amount absorbed at 24 hours does not include receptor wash.

Table 18: Butylparaben – human – concentration-time course

Time (hours)	Radioactivity (µg equiv/mL)		Butylparaben (µg/mL)		4-hyroxybenzoic acid (µg/mL)	
	Mean	SD	Mean	SD	Mean	SD
1	0.09	0.07	0.04	0.04	0.04	0.03
2	0.79	0.35	0.59	0.41	0.21	0.10
3	1.09	0.29	0.77	0.60	0.25	0.08
4	0.90	0.20	0.61	0.33	0.26	0.09
5	0.76	0.20	0.44	0.23	0.23	0.06
6	0.72	0.25	0.34	0.25	0.19	0.06
8	0.53	0.17	0.23	0.14	0.16	0.06
10	0.48	0.16	0.37	0.31	0.17	0.10
12	0.39	0.13	0.23	0.06	0.13	0.05
14	0.38	0.12	0.18	0.06	0.12	0.05
16	0.34	0.09	0.12	0.05	0.13	0.04
18	0.28	0.06	0.10	0.04	0.12	0.05
20	0.26	0.07	0.08	0.03	0.10	0.05
22	0.25	0.06	0.07	0.02	0.10	0.05
24	0.22	0.06	0.05	0.03	0.08	0.05

Table 19: Butylparaben – human – cumulative percent absorbed

Time	Radioactivity		Butylparaben		4-hyroxybenzoic acid	
(hours)	Mean	SD	Mean	SD	Mean	SD
1	0.61	0.49	0.29	0.28	0.28	0.21
2	6.16	2.94	4.38[a]	3.03	1.76[a]	0.89
3	13.79	4.68	9.69[a]	6.74	3.49[a]	1.43
4	20.03	5.15	13.95[a]	8.53	5.28[a]	1.97
5	25.31	5.35	17.03[a]	9.43	6.85[a]	2.31
6	30.29[a]	5.62	19.40[a]	10.10	8.18[a]	2.56
8	37.68[a]	6.63	22.61[a]	11.04	10.46[a]	3.19
10	44.85[a]	7.10	31.13[a]	14.18	11.37[a]	3.77
12	50.34[a]	8.21	34.27[a]	14.23	13.18[a]	4.39
14	55.67[a]	8.72	36.75[a]	14.30	14.92[a]	4.86
16	59.36[a]	9.40	32.40[a]	14.27	18.48[a]	5.47
18	63.34[a]	9.70	33.77[a]	14.48	20.10	6.06
20	66.95[a]	10.11	34.90[a]	14.59	21.56	6.64
22	70.45[a]	10.30	35.83[a]	14.73	22.97	7.20
24[b]	73.51[a]	10.34	36.57[a]	14.81	24.08	7.59

[a] Statistically different from butylparaben rat ($p < 0.05$).
[b] Cumulative amount absorbed at 24 hours does not include receptor wash.

Table 20: Butylparaben – human – material balance

		Mean	SD
Absorbed dose			
	Receptor fluid	73.51	10.34
	Receptor wash	0.72	0.21
	Total absorbed	74.23	10.32
Absorbable dose			
	Receptor fluid	73.51	10.34
	Receptor wash	0.72	0.21
	Skin	6.92	1.77
	Total absorbable	81.15	10.65
Unabsorbed dose			
	Skin wash	15.65	8.29
	Donor chamber	0.60	0.60
	Tape strips	1.41	1.11
	Total unabsorbed	17.66	9.38
Total recovered		98.81	8.65

FIGURES

FIGURES

EXPLANATORY NOTES

ABBREVIATIONS:

CPM	count per minute
DPM	disintegrations per minute
Ethyl Paraben	ethylparaben
EtOH	ethanol
h	hours
Methyl Paraben	methylparaben
n-Butyl Paraben	butylparaben
para-Hydroxybenzoic Acid	4-hydroxybenzoic acid

Figure 1: ^{14}C-methylparaben, Flo-one radiochromatogram, neat

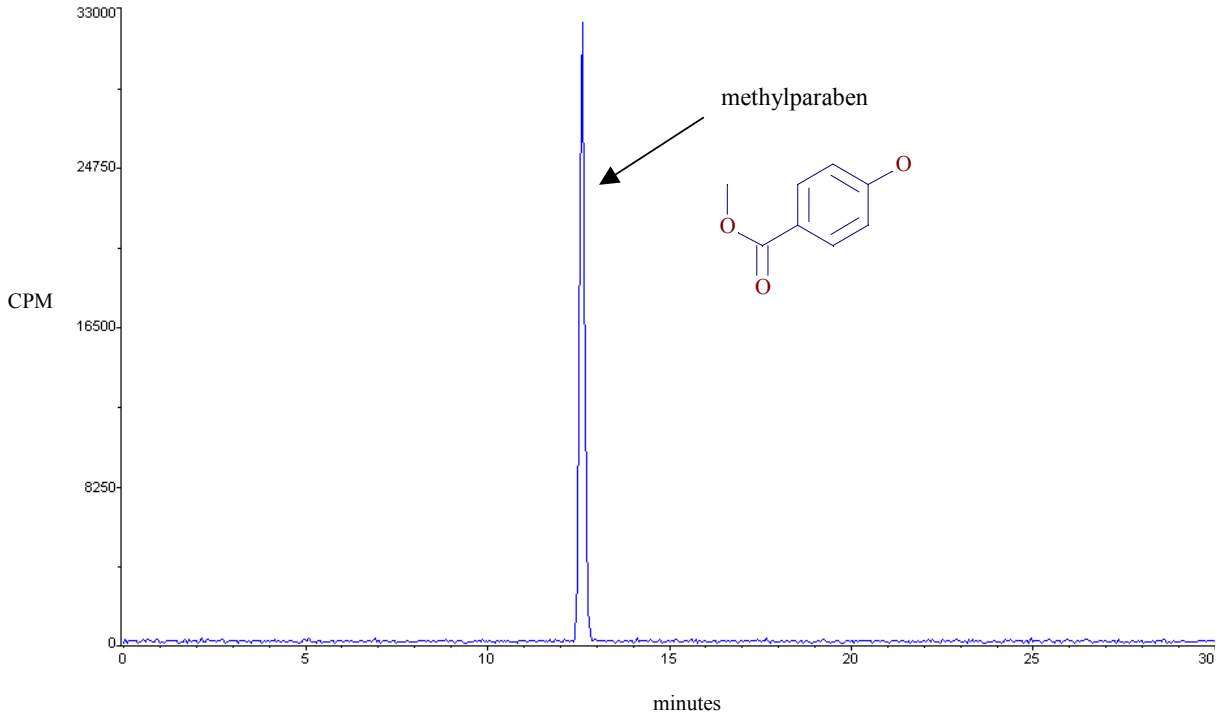


Figure 2: ^{14}C-butylparaben, Flo-one radiochromatogram, neat

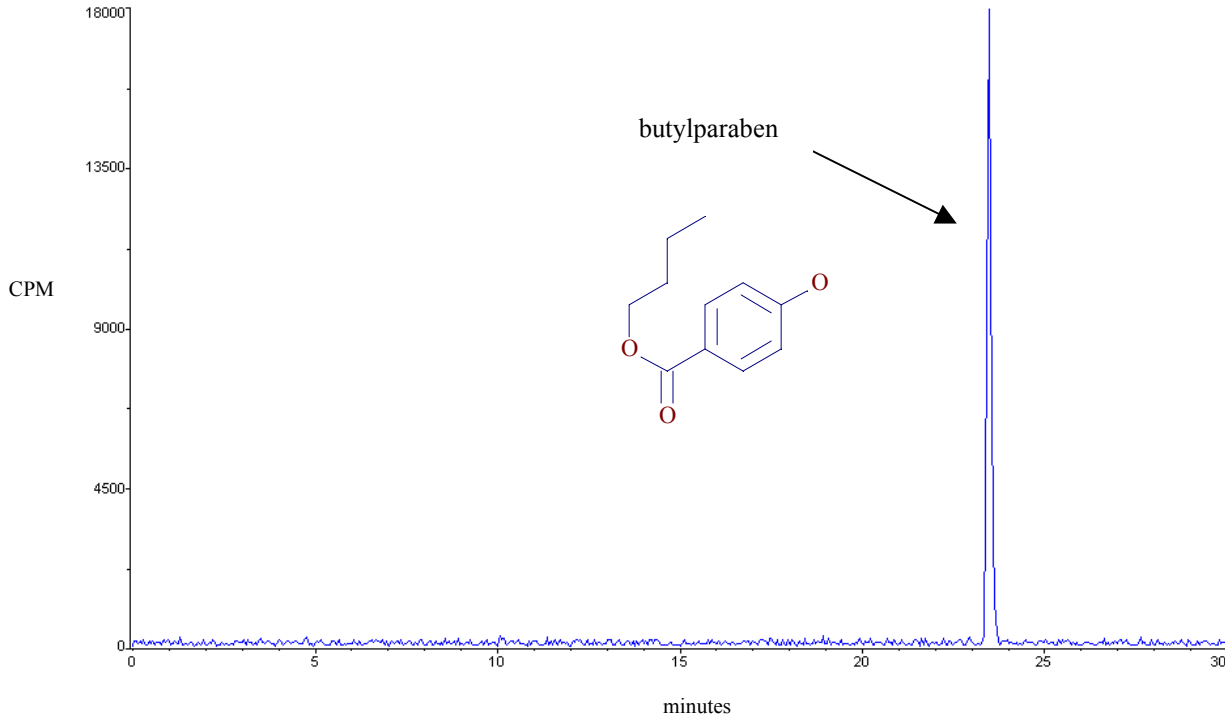


Figure 3: Representative radiochromatogram ^{14}C-methylparaben in 0.8% emulsion

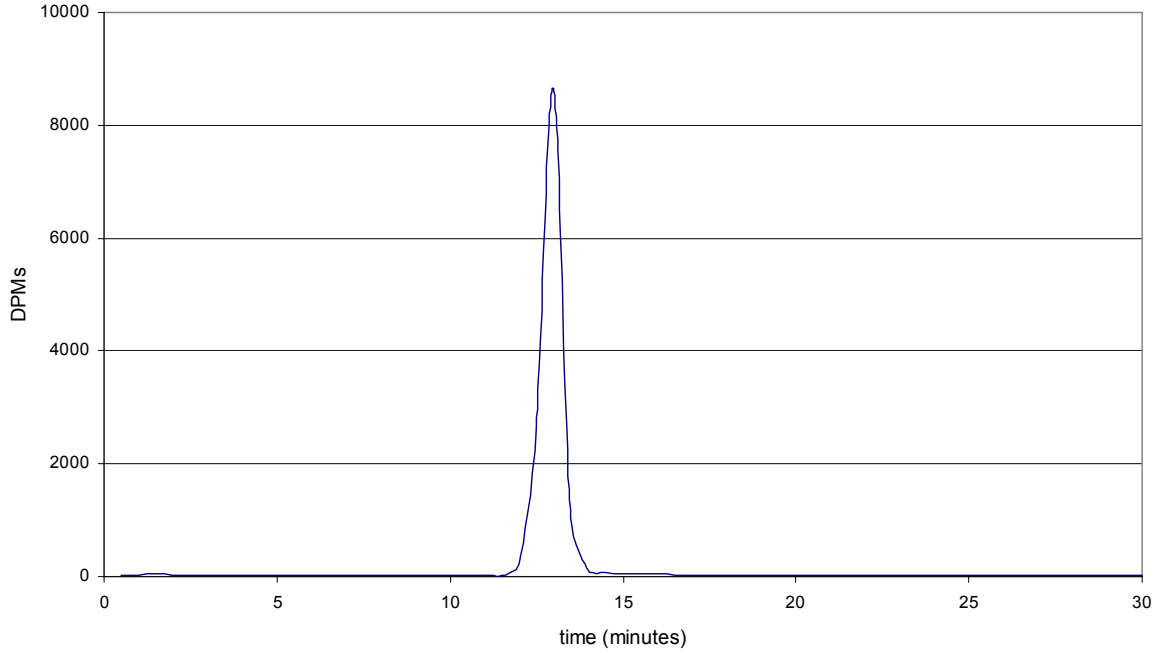


Figure 4: Representative radiochromatogram ^{14}C-butylparaben in 0.4% emulsion

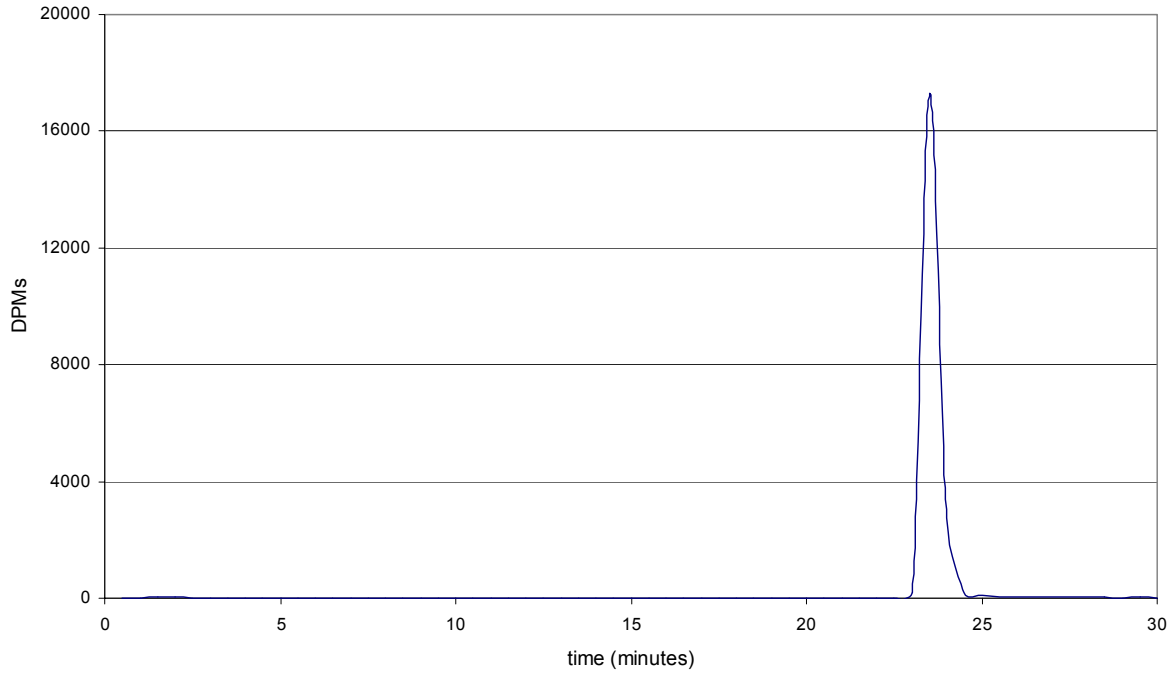


Figure 5: Methylparaben – rat – cumulative amount absorbed per area, total radioactivity, methylparaben, 4-hydroxybenzoic acid

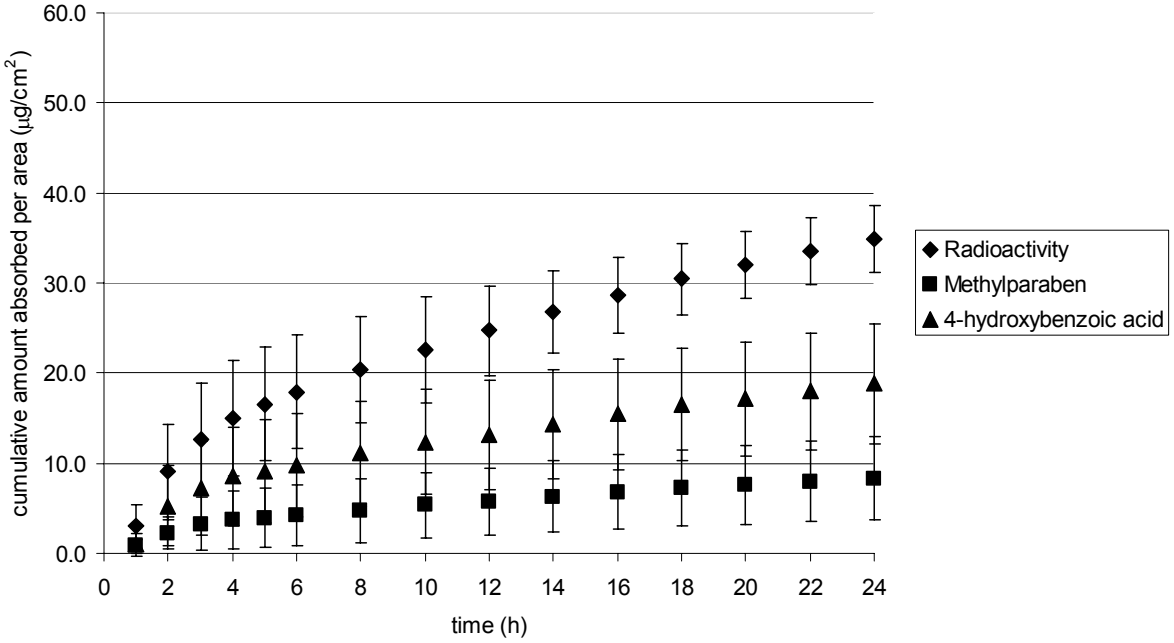


Figure 6: Methylparaben – rat – concentration-time course, total radioactivity, methylparaben, 4-hydroxybenzoic acid

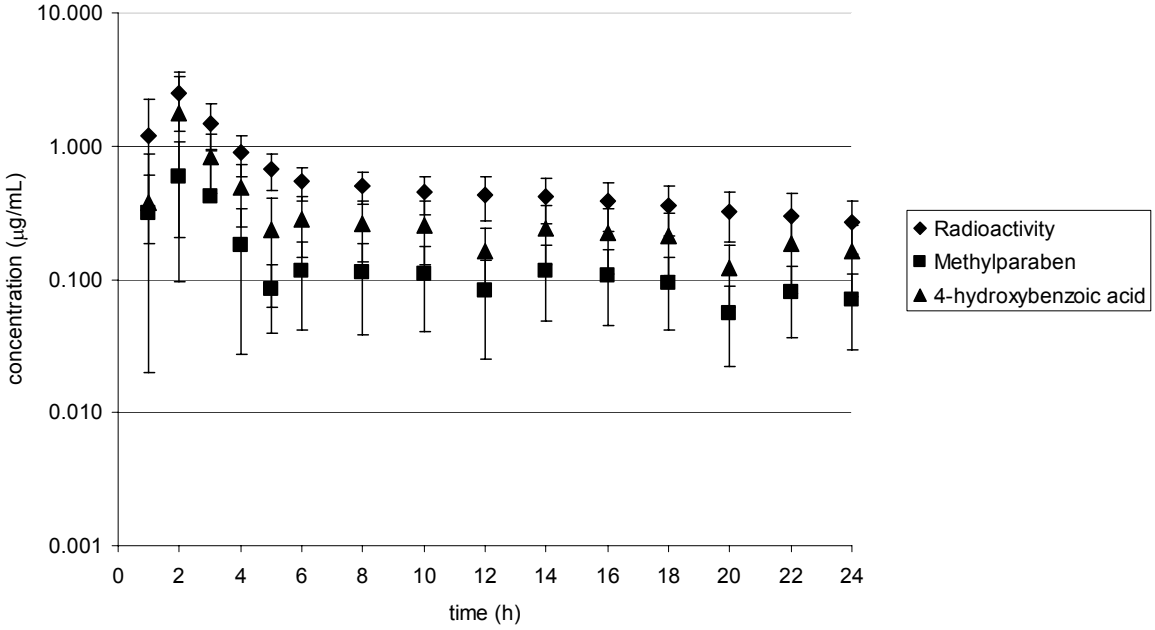


Figure 7: Methylparaben – rat – cumulative percent absorbed, total radioactivity, methylparaben, 4-hydroxybenzoic acid

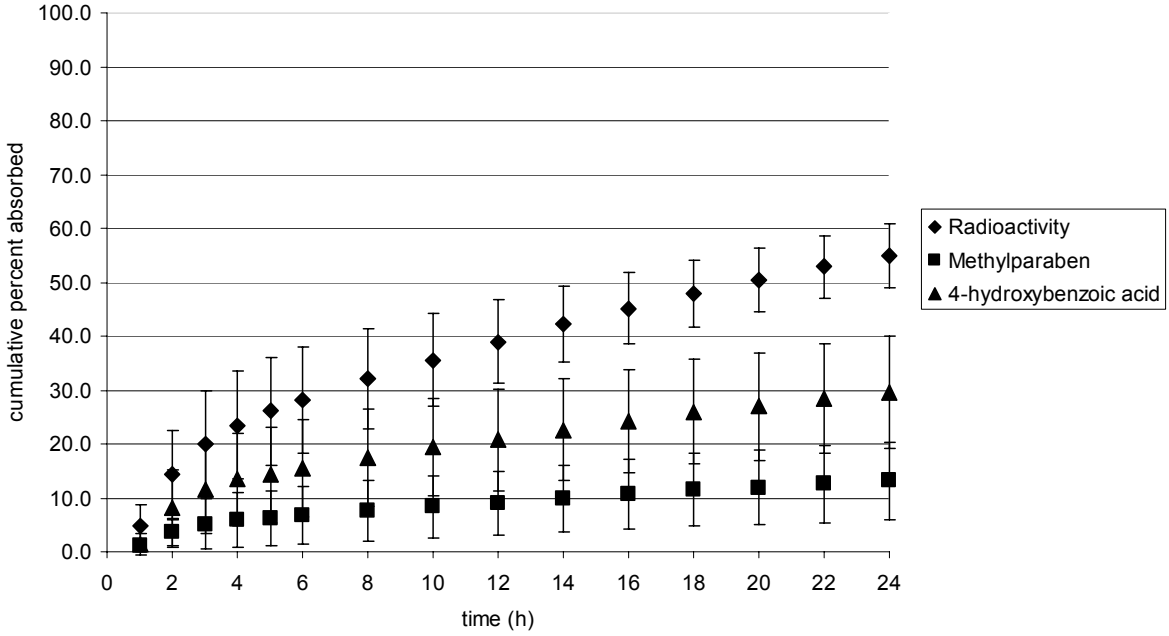


Figure 8: Methylparaben – rat – total radioactivity, material balance

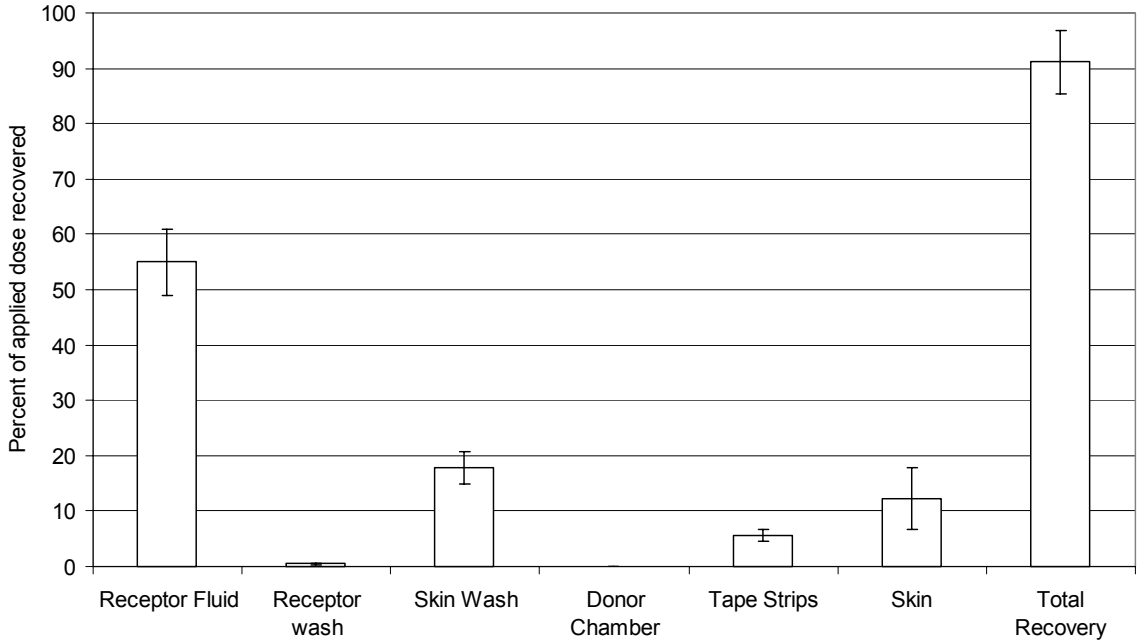


Figure 9: Methylparaben – rat – absorbed, absorbable, unabsorbed, total recovered

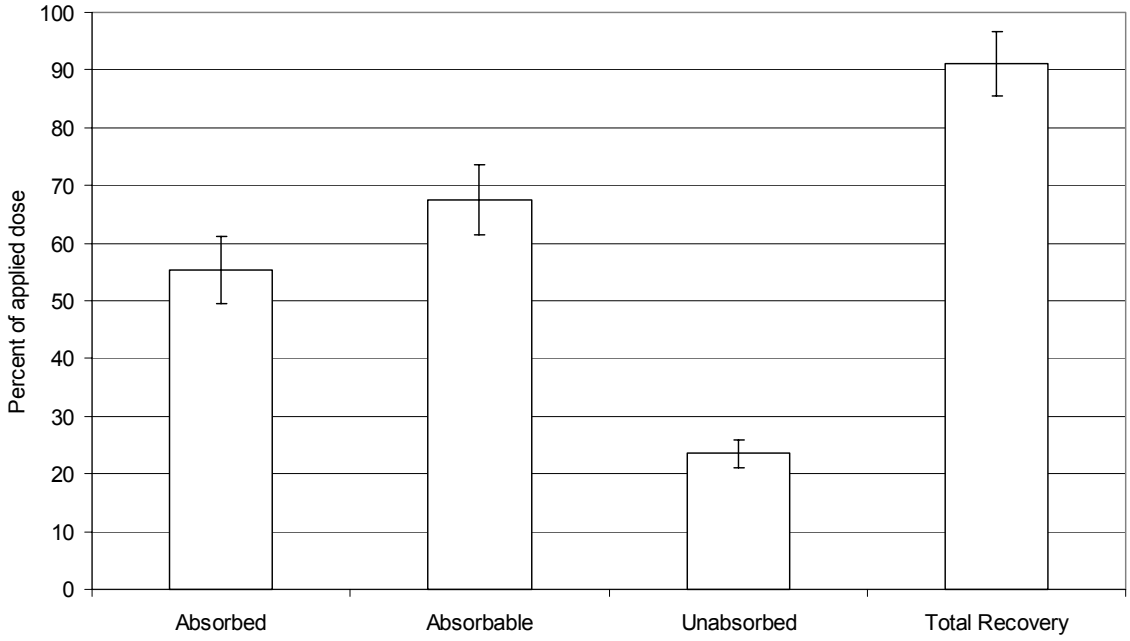


Figure 10: Methylparaben – human – cumulative amount absorbed per area, total radioactivity, methylparaben, 4-hydroxybenzoic acid

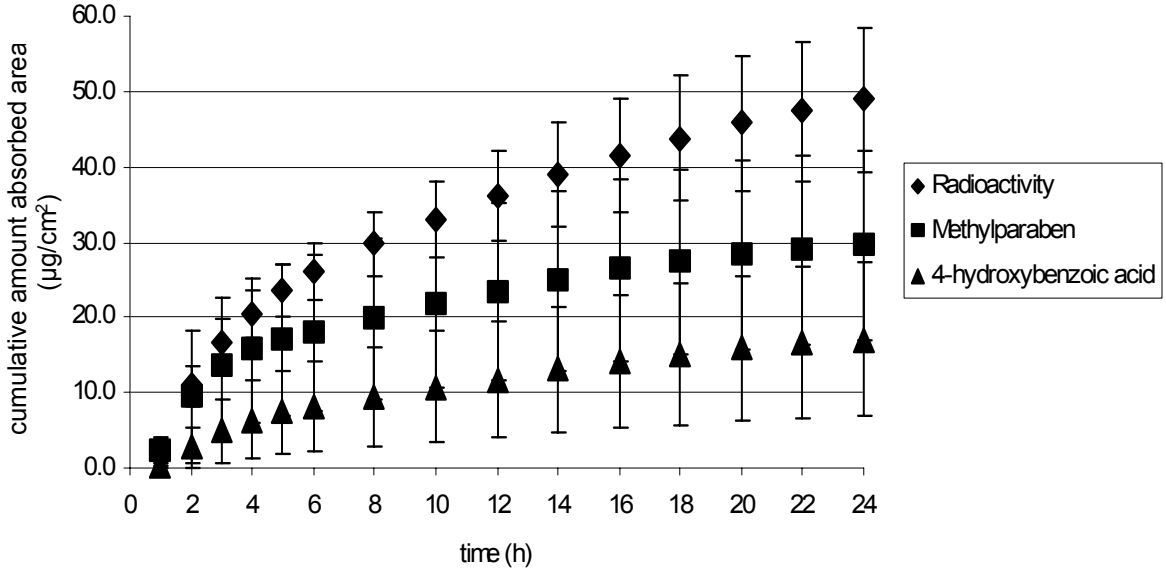


Figure 11: Methylparaben – human – concentration-time course, total radioactivity, methylparaben, 4-hydroxybenzoic acid

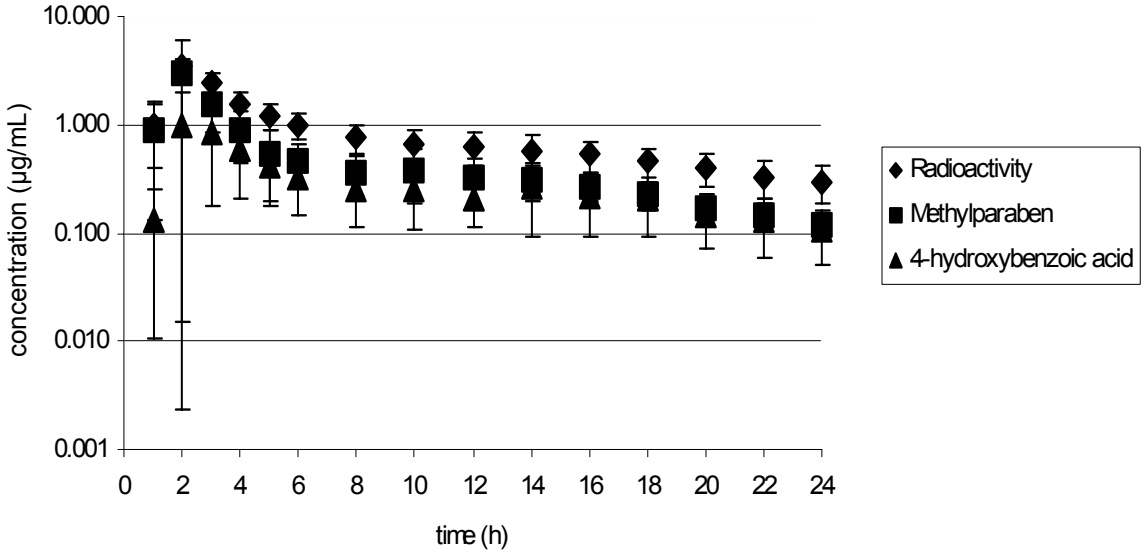


Figure 12: Methylparaben – human – cumulative percent absorbed, total radioactivity, methylparaben, 4-hydroxybenzoic acid

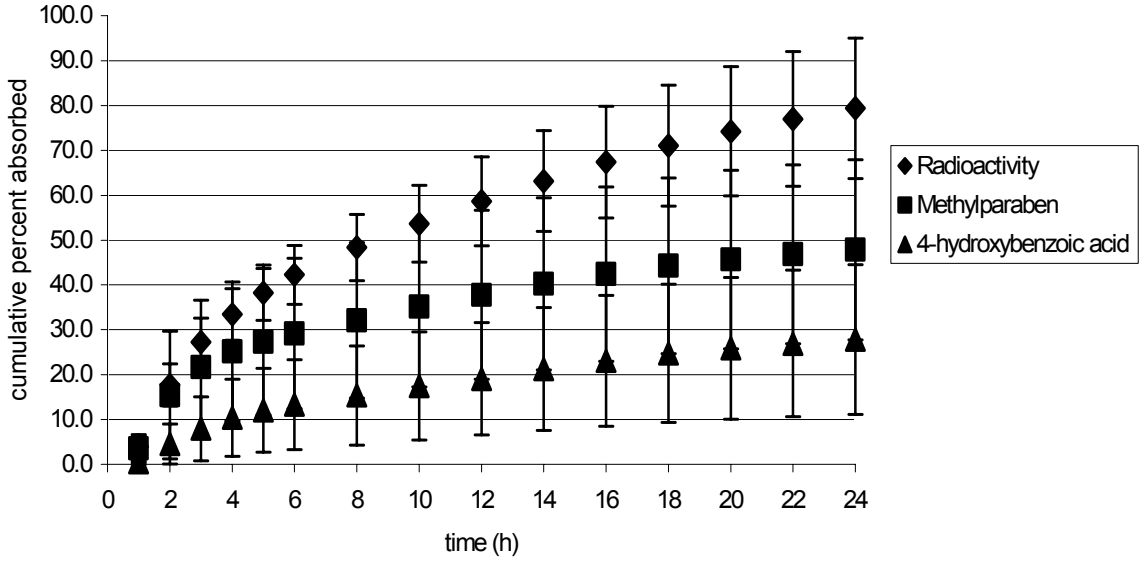


Figure 13: Methylparaben – human – total radioactivity, material balance

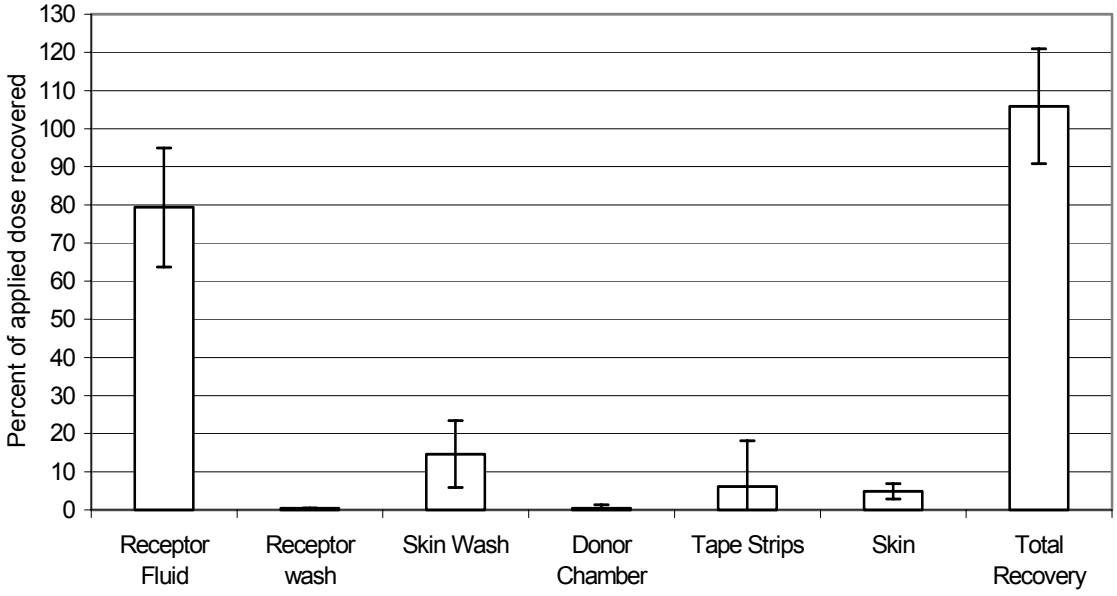


Figure 14: Methylparaben – human – absorbed, absorbable, unabsorbed, total recovered

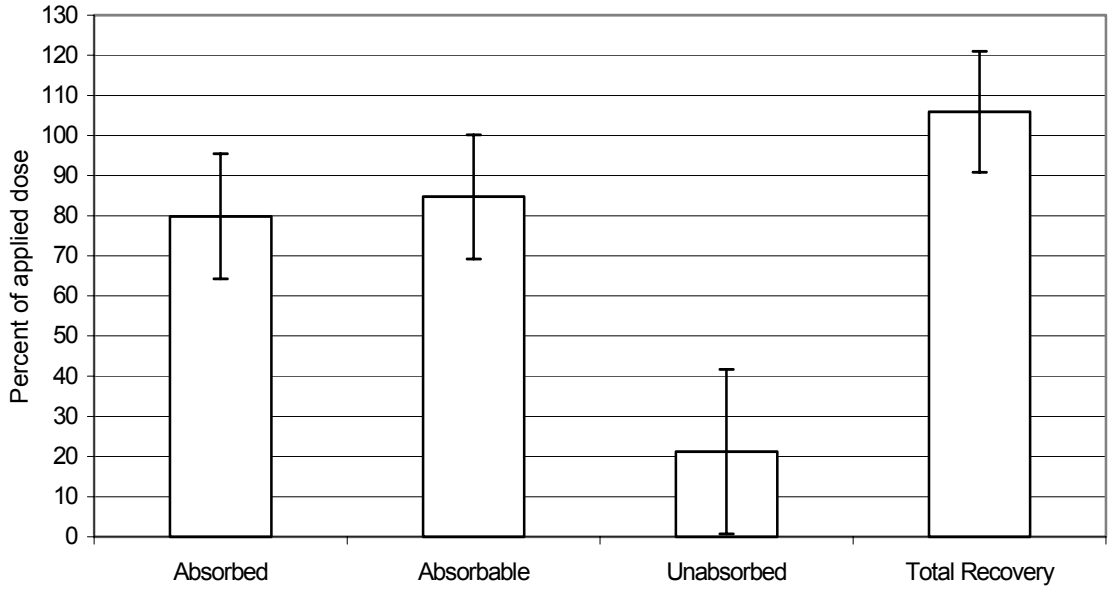


Figure 15: Butylparaben – rat – cumulative amount absorbed per area, total radioactivity, butylparaben, 4-hydroxybenzoic acid

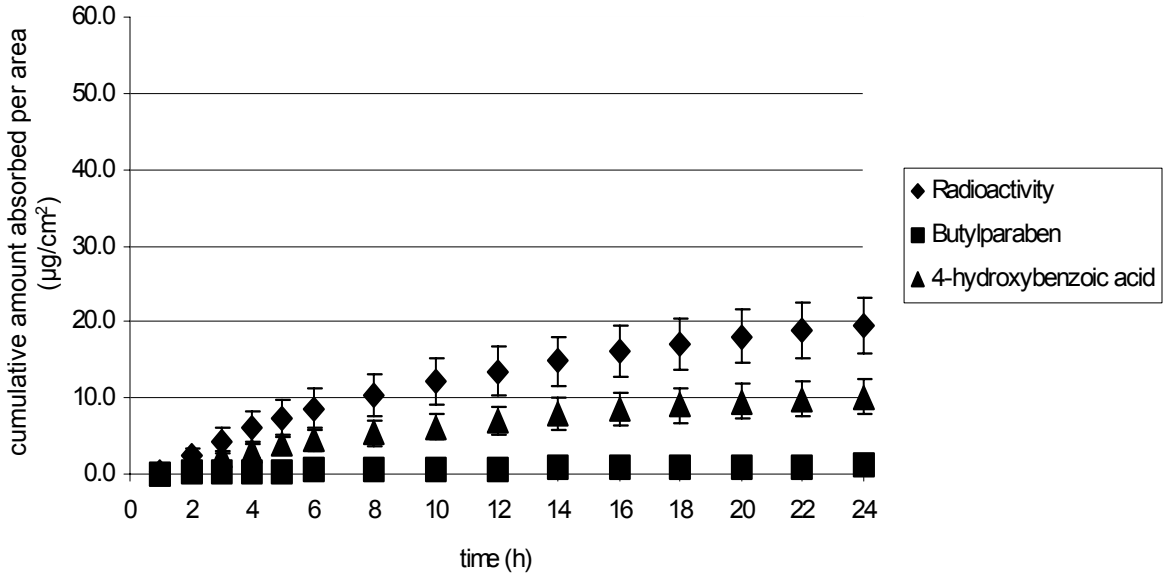


Figure 16: Butylparaben – rat – concentration-time course, total radioactivity, butylparaben, 4-hydroxybenzoic acid

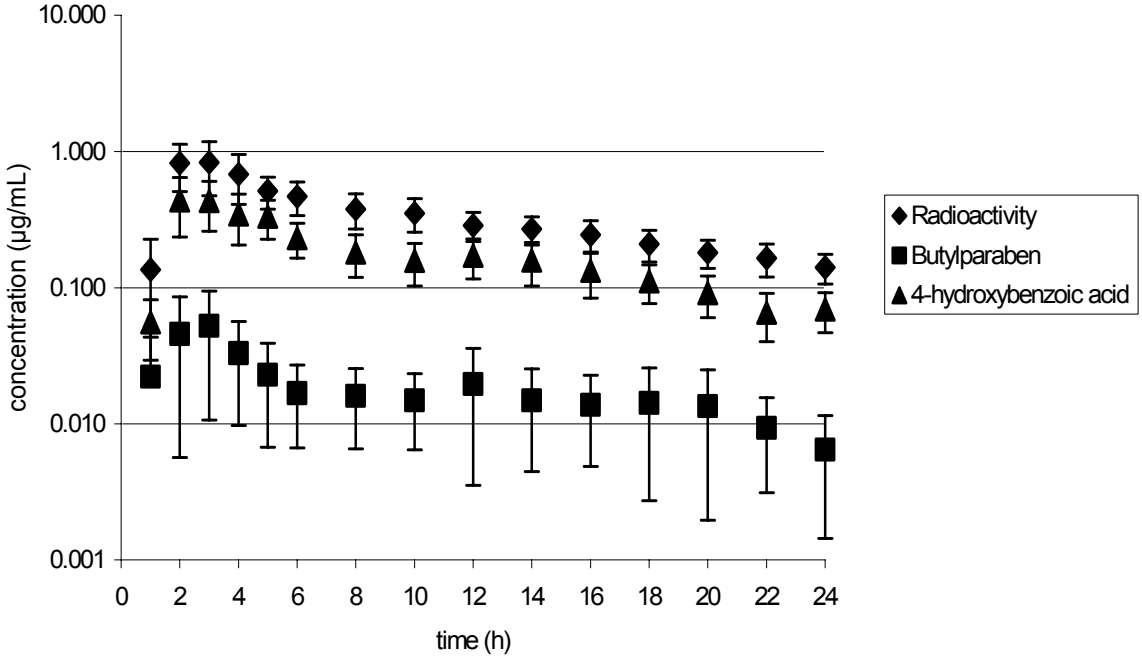


Figure 17: Butylparaben – rat – cumulative percent absorbed, total radioactivity, butylparaben, 4-hydroxybenzoic acid

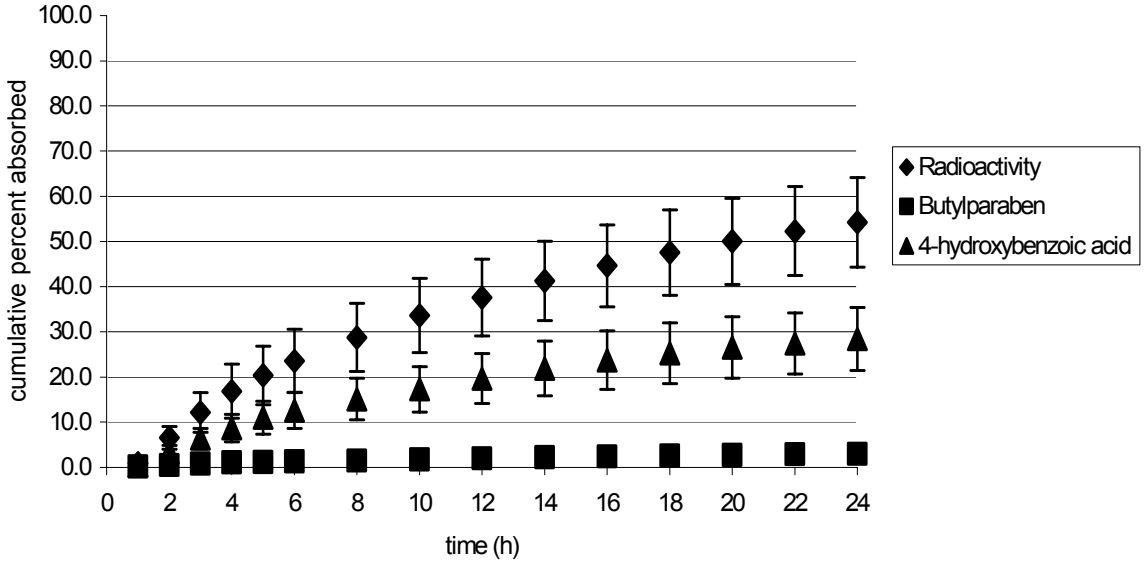


Figure 18: Butylparaben – rat – total radioactivity, material balance

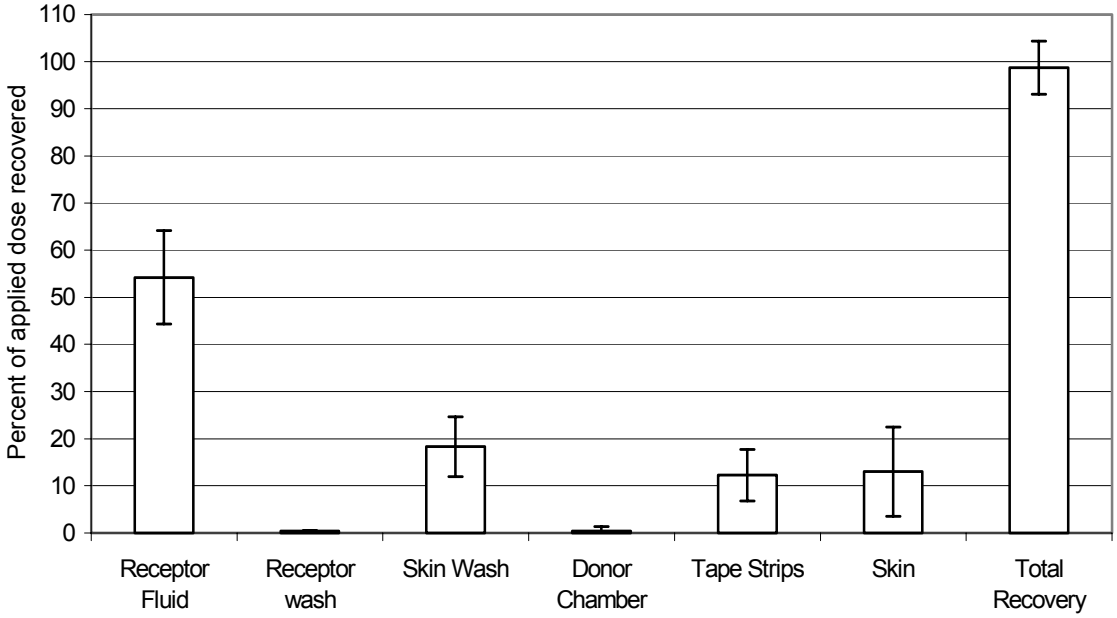


Figure 19: Butylparaben – rat – absorbed, absorbable, unabsorbed, total recovered

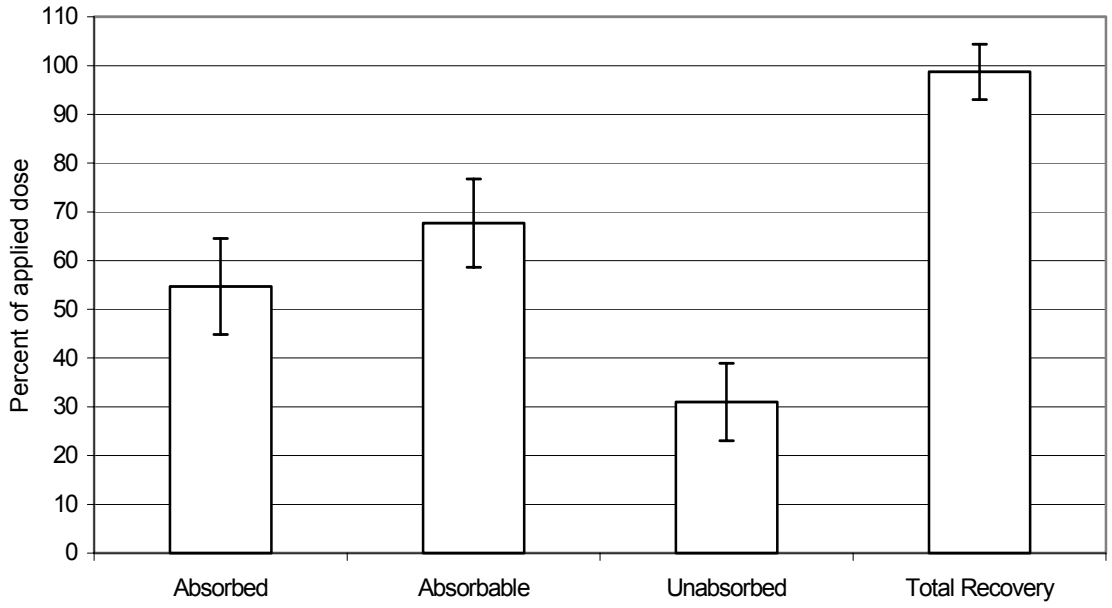


Figure 20: Butylparaben – human – cumulative amount absorbed per area, total radioactivity, butylparaben, 4-hydroxybenzoic acid

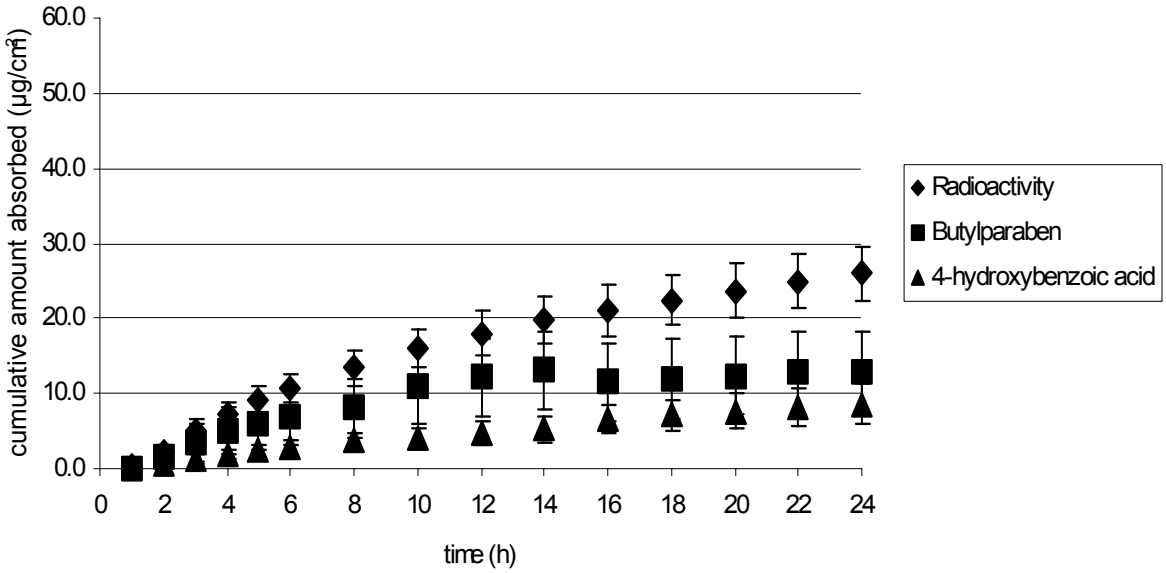


Figure 21: Butylparaben – human – concentration-time course, total radioactivity, butylparaben, 4-hydroxybenzoic acid

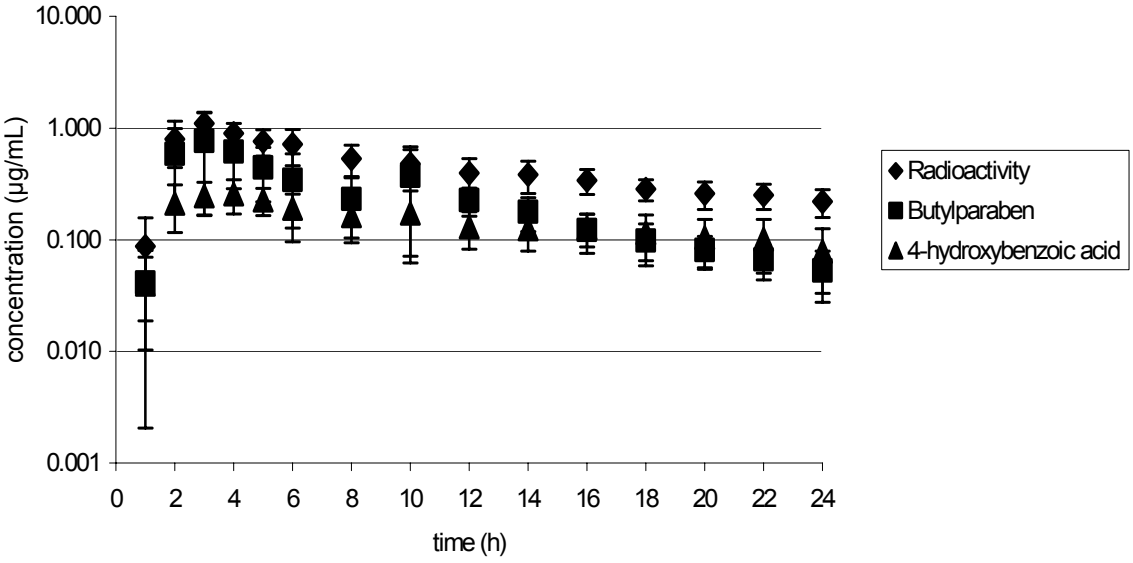


Figure 22: Butylparaben – human – cumulative percent absorbed, total radioactivity, butylparaben, 4-hydroxybenzoic acid

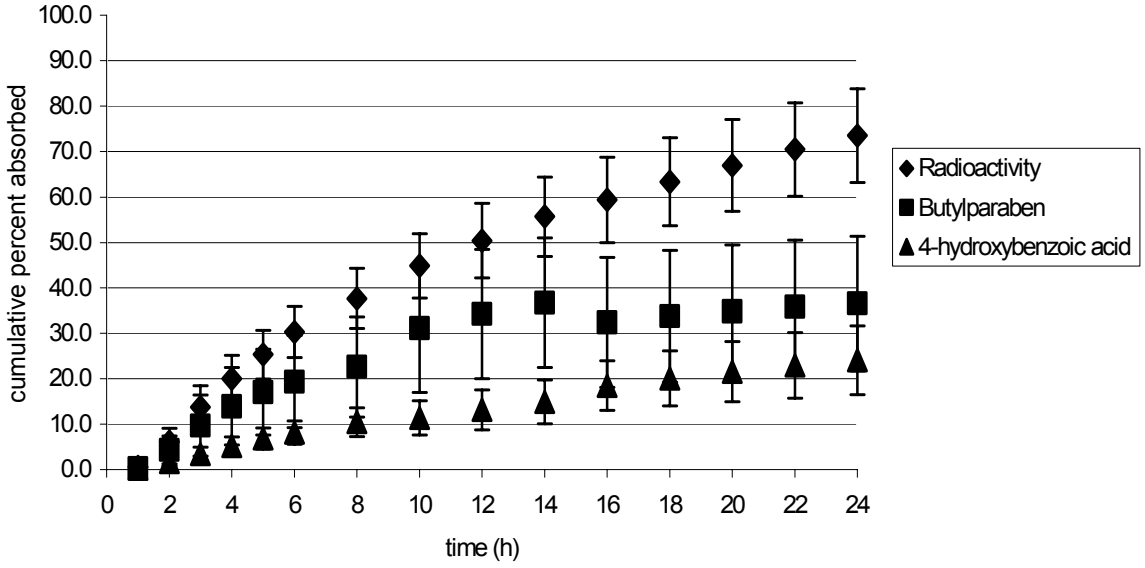


Figure 23: Butylparaben – human – total radioactivity, material balance

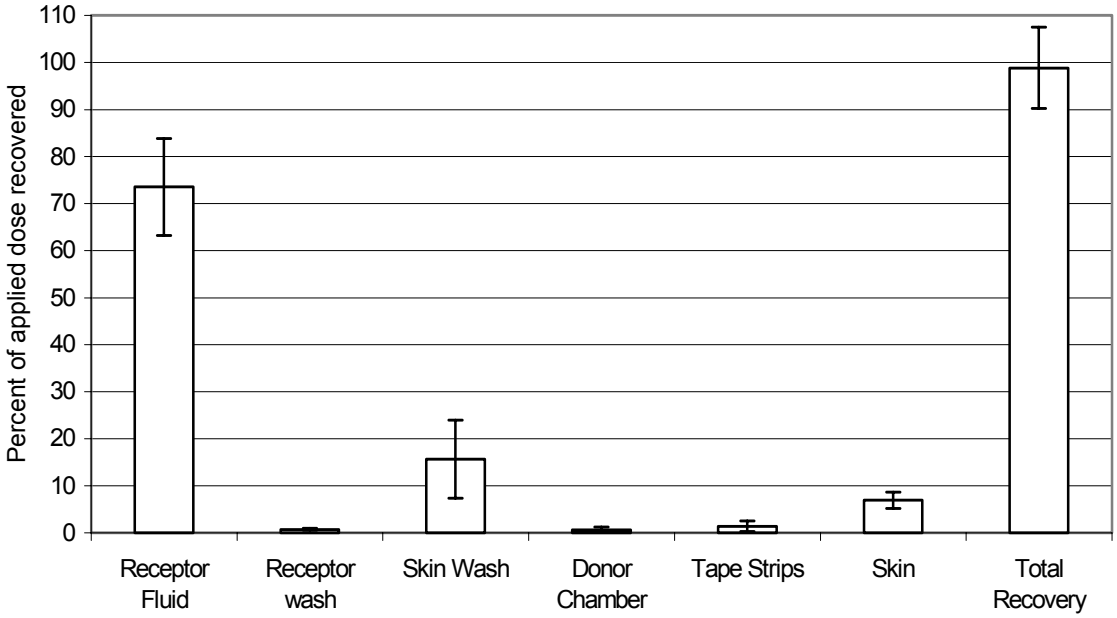


Figure 24: Butylparaben – human – absorbed, absorbable, unabsorbed, total recovered

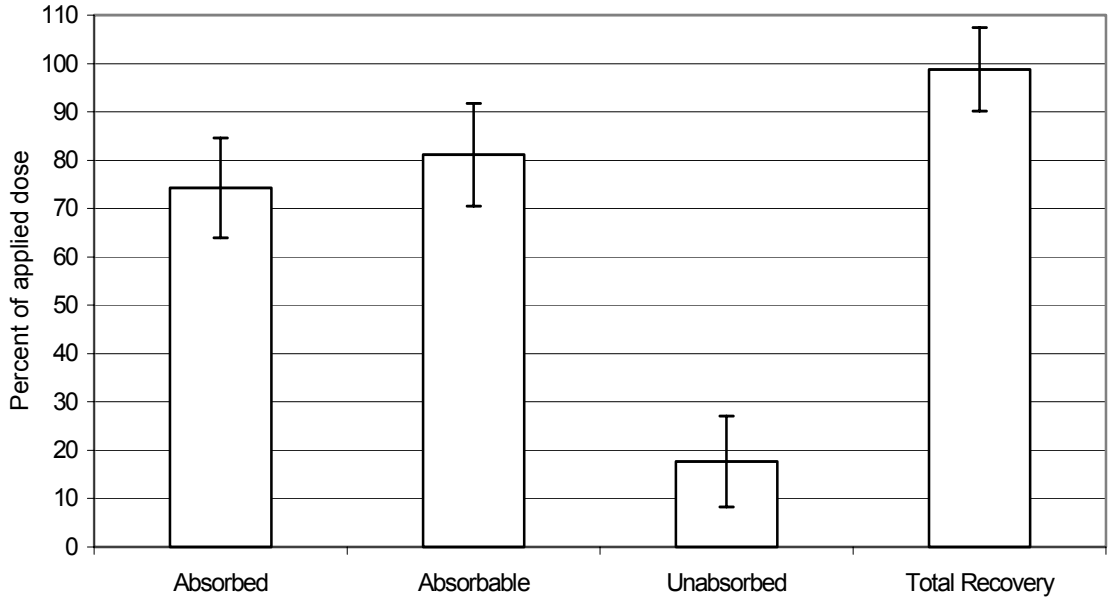


Figure 25: Methylparaben – rat – representative radiochromatogram of receptor fluid pool

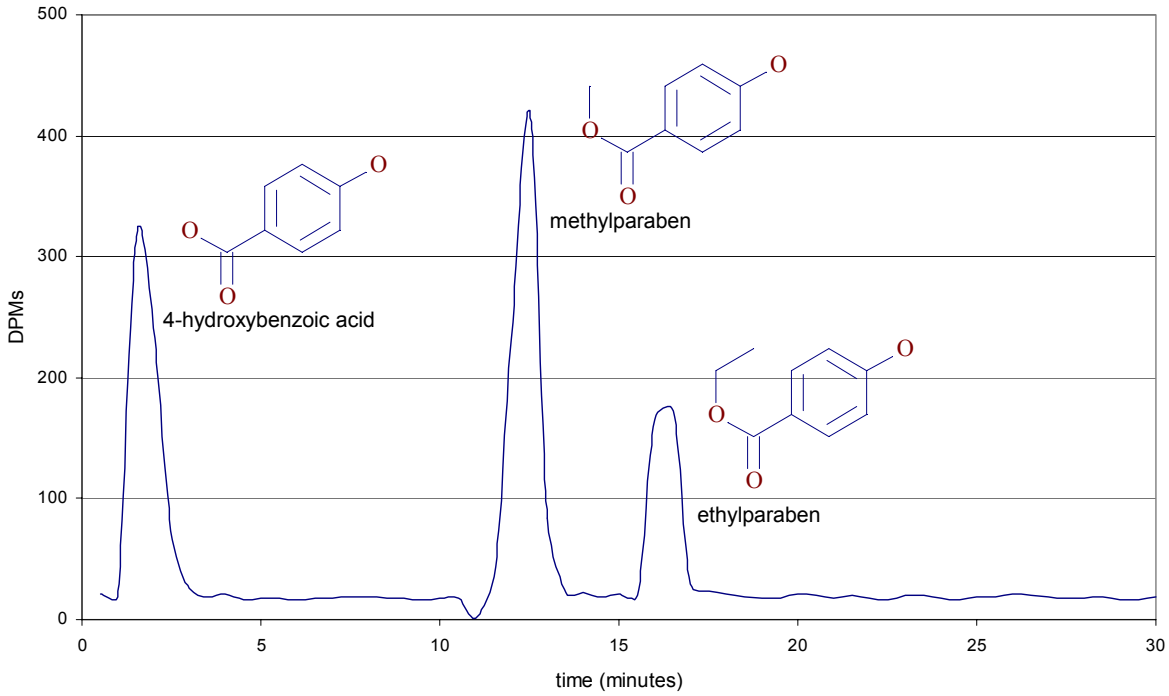


Figure 26: Methylparaben – human – representative radiochromatogram of receptor fluid pool

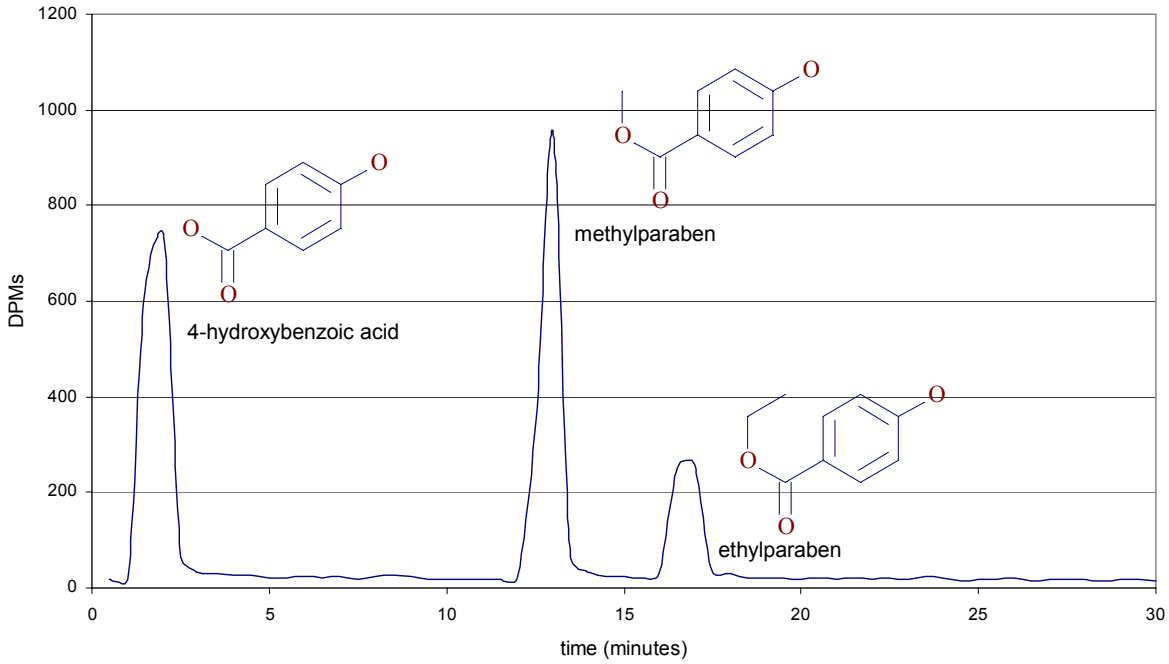


Figure 27: Butylparaben – rat – representative radiochromatogram of receptor fluid pool

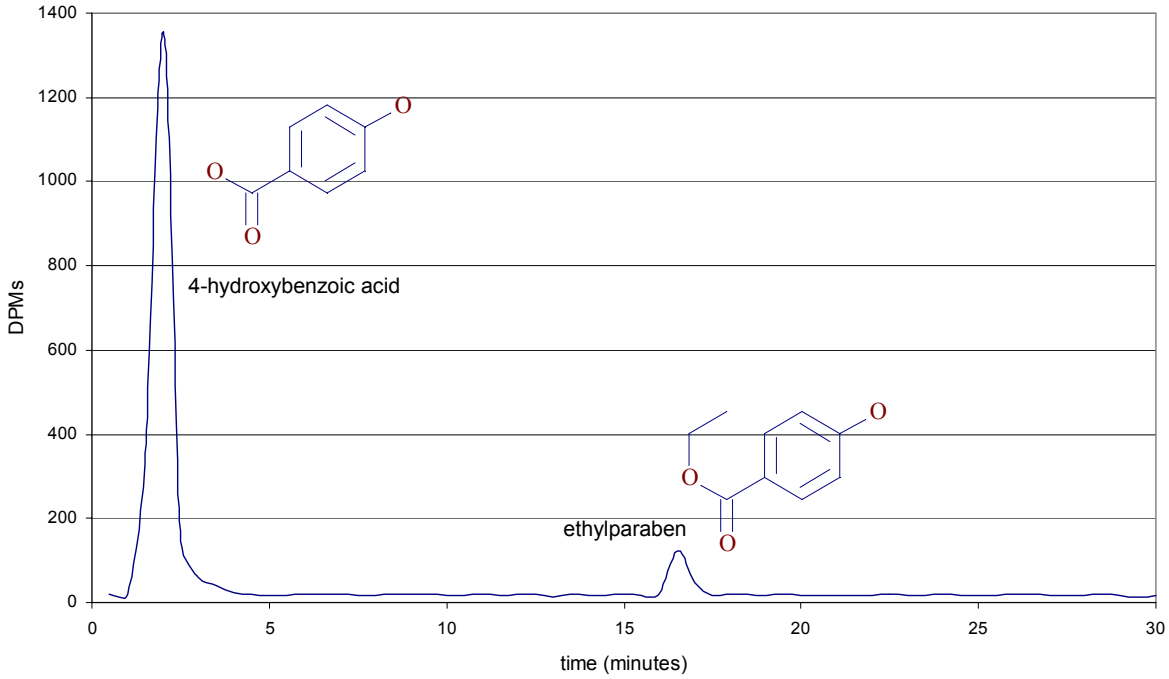


Figure 28: Butylparaben – human – representative radiochromatogram of receptor fluid pool

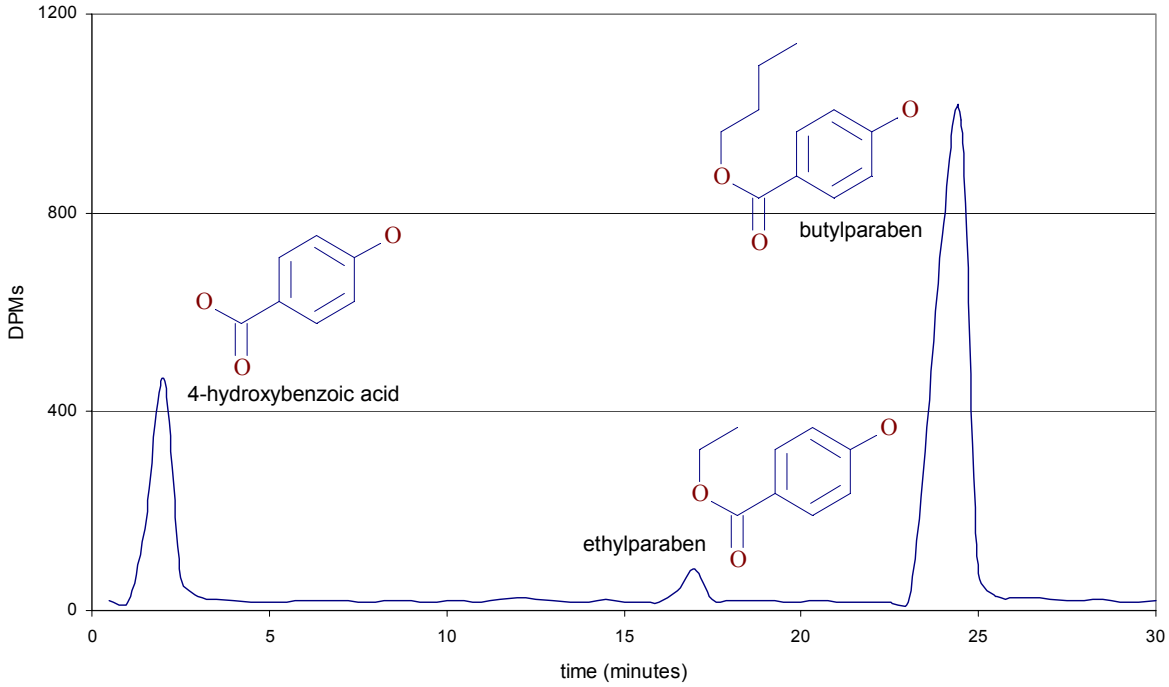


Figure 29: Negative electrospray ionization mass spectrum of unknown with a retention time of 2.5 minutes from the representative methylparaben receptor fluid pool (4-hydroxybenzoic acid)

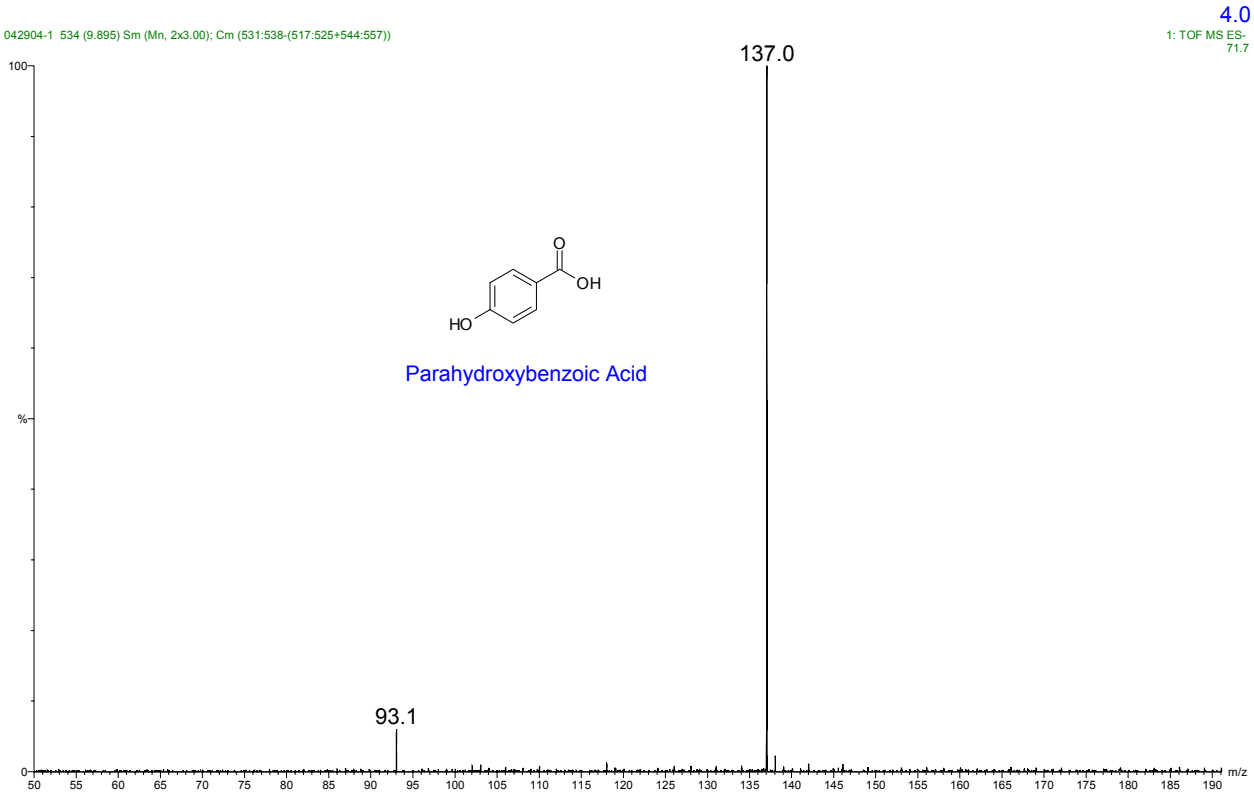


Figure 30: Negative electrospray ionization daughter ion mass spectrum of unknown with a retention time of 2.5 minutes from the representative methylparaben receptor fluid pool (4-hydroxybenzoic acid)

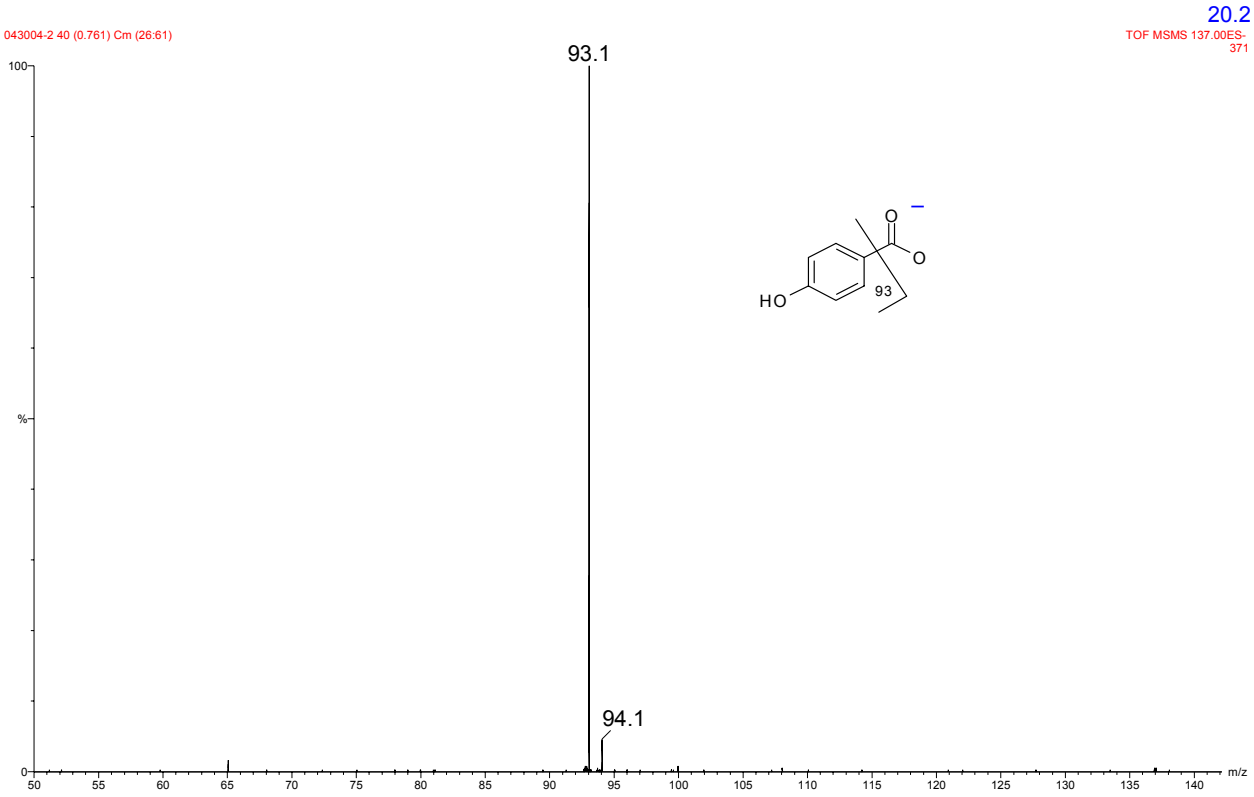


Figure 31: Negative electrospray ionization mass spectrum of unknown with a retention time of 12.5 minutes from the representative methylparaben receptor fluid pool (methylparaben)

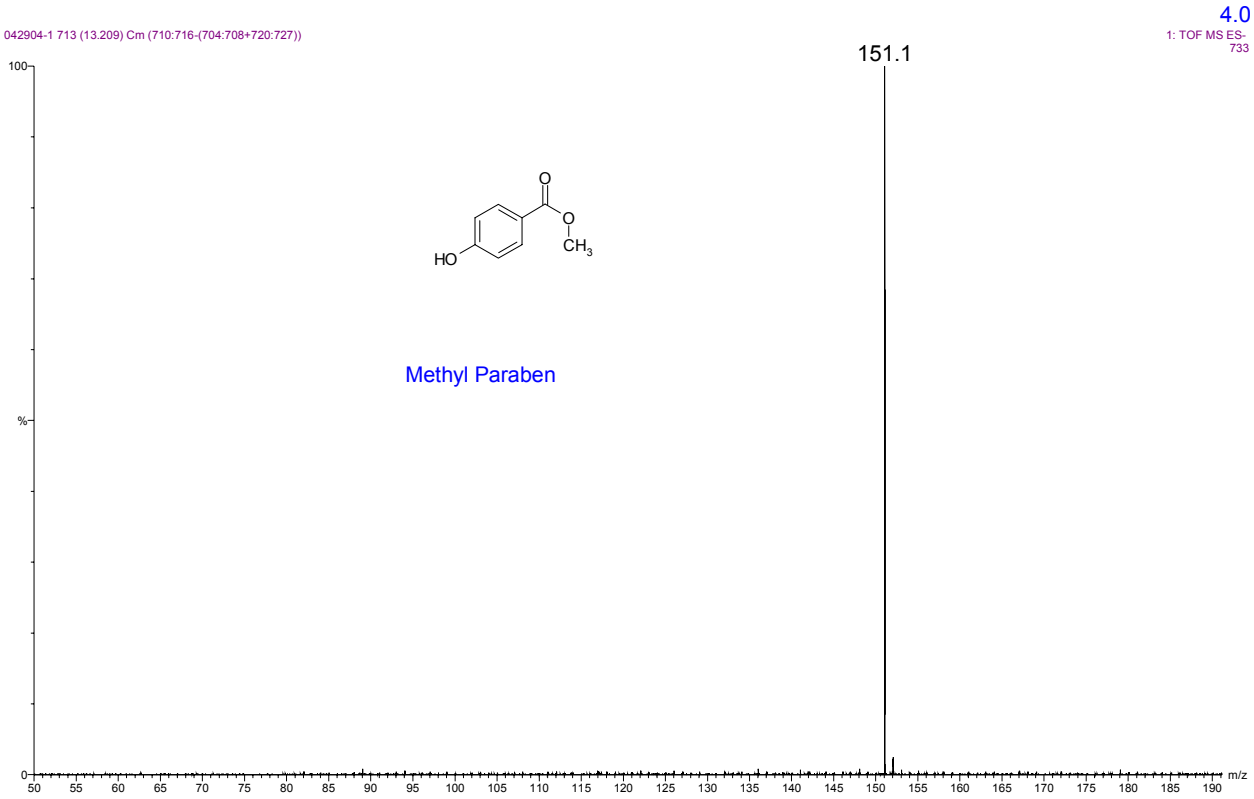


Figure 32: Negative electrospray ionization daughter ion mass spectrum of unknown with a retention time of 12.5 minutes from the representative methylparaben receptor fluid pool (methylparaben)

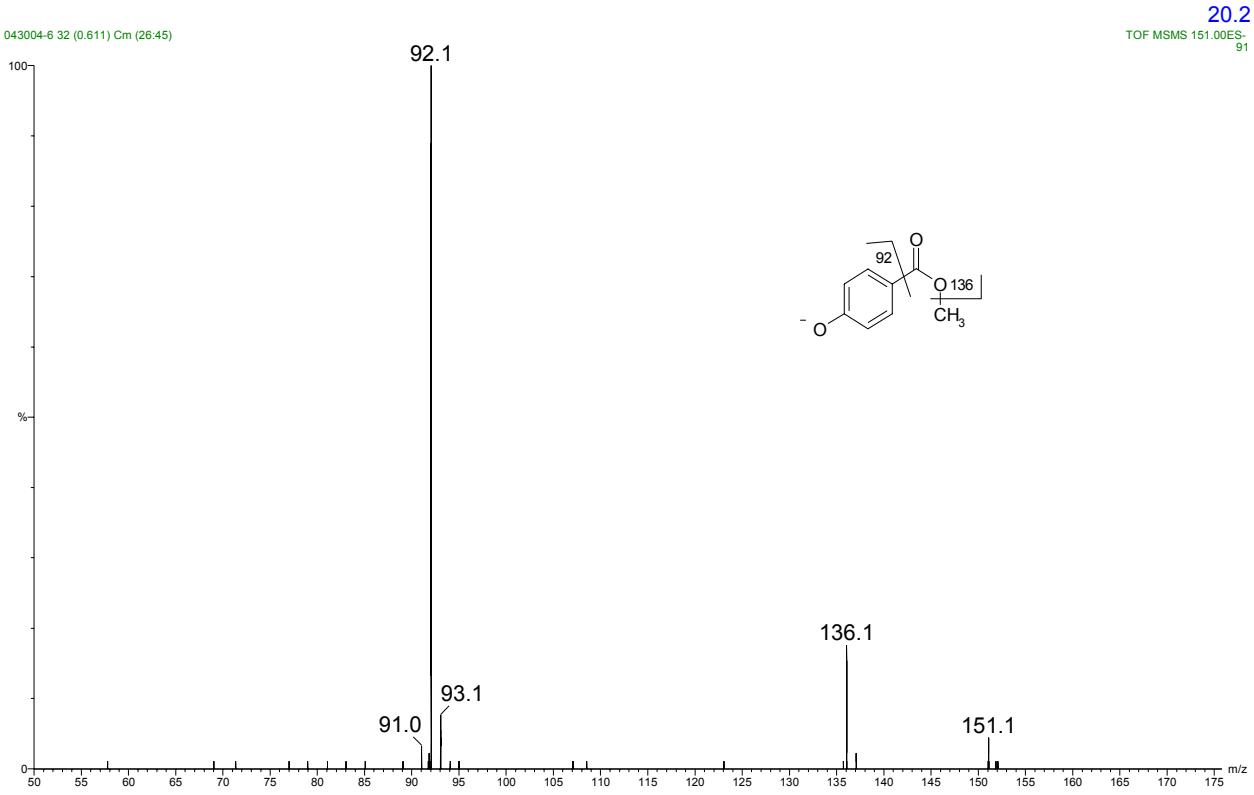


Figure 33: Negative electrospray ionization mass spectrum of unknown with a retention time of 17.5 minutes from the representative methylparaben receptor fluid pool (ethylparaben)

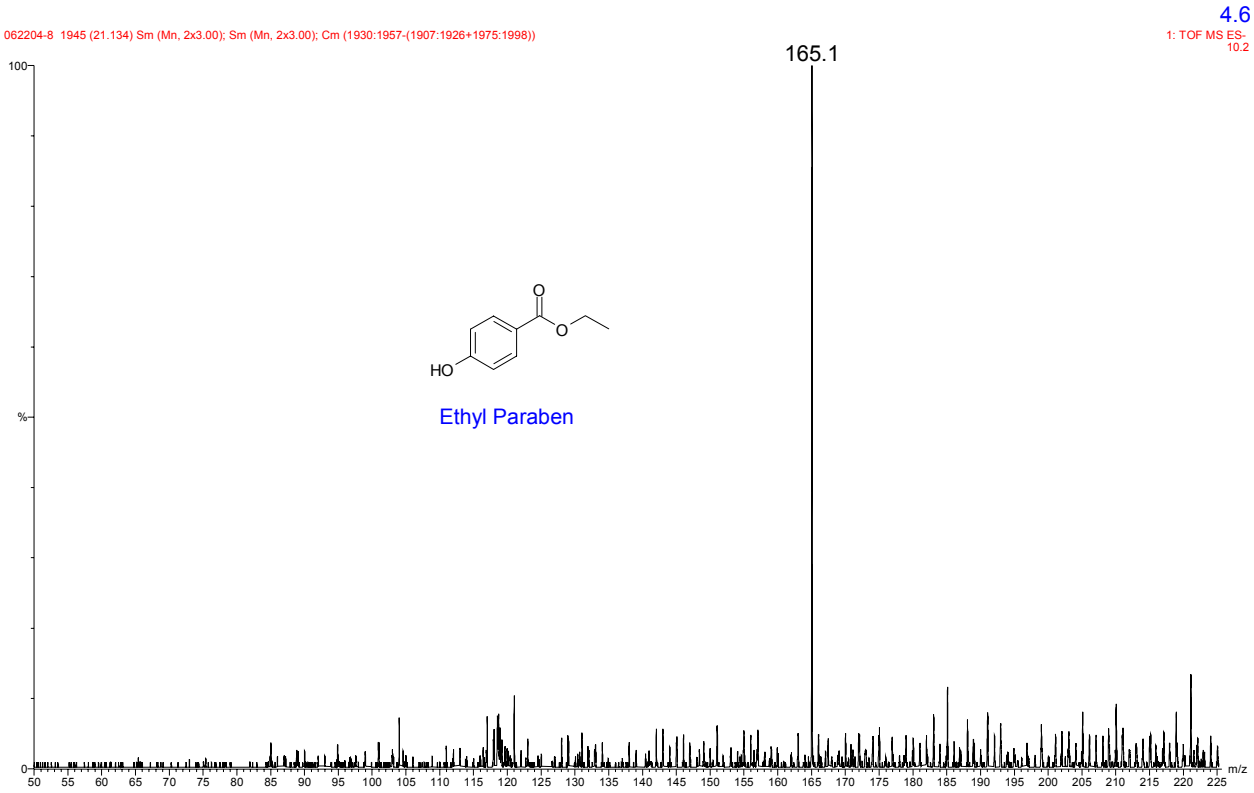


Figure 34: Negative electrospray ionization daughter ion mass spectrum of unknown with a retention time of 17.5 minutes from the representative methylparaben receptor fluid pool (ethylparaben)

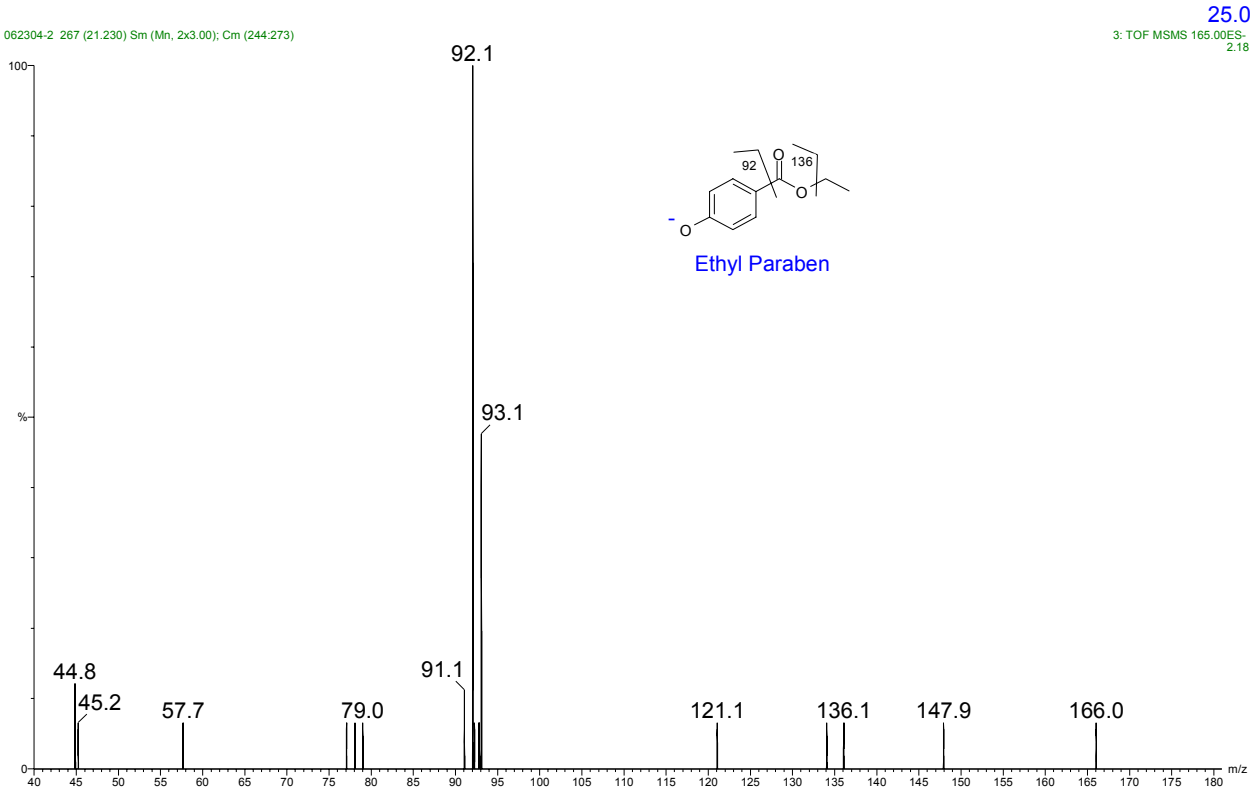


Figure 35: Negative electrospray ionization mass spectrum of unknown with a retention time of 24.5 minutes from the representative butylparaben receptor fluid pool (butylparaben)

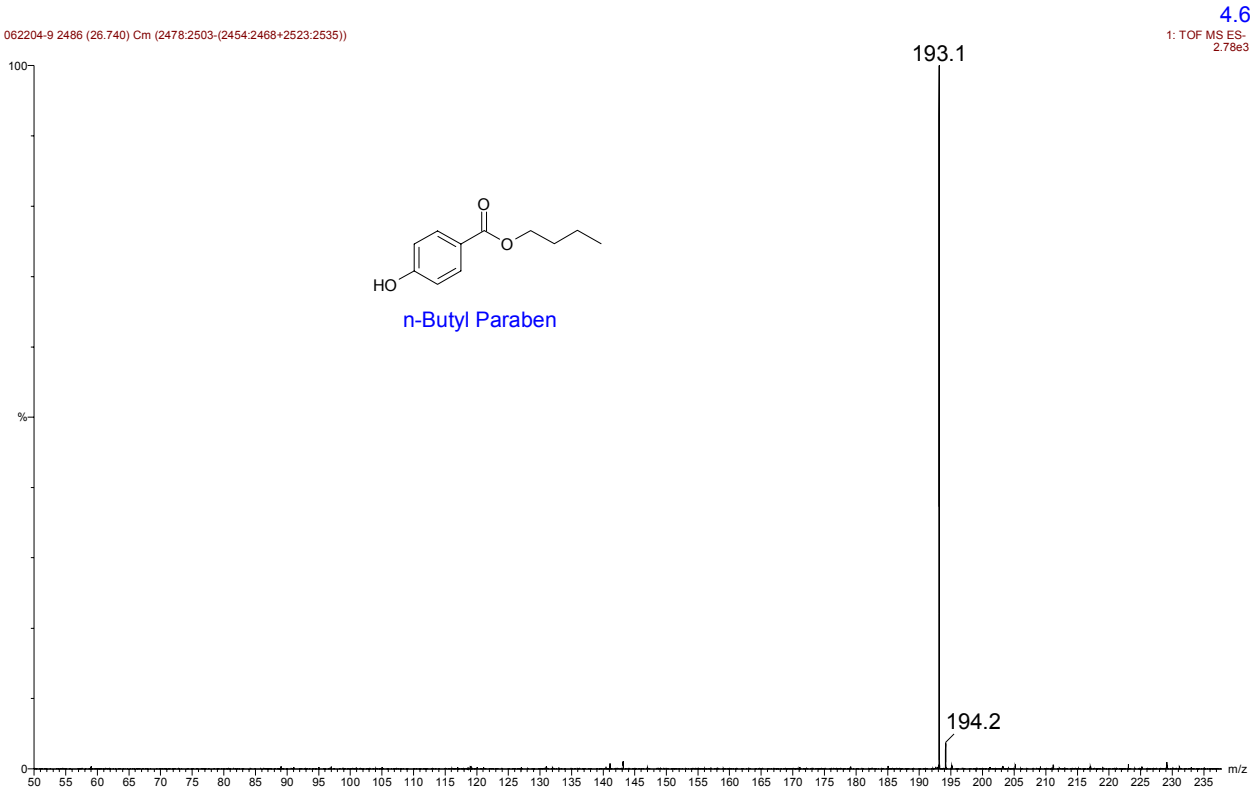


Figure 36: Negative electrospray ionization daughter ion mass spectrum of unknown with a retention time of 24.5 minutes from the representative butylparaben receptor fluid pool (butylparaben)

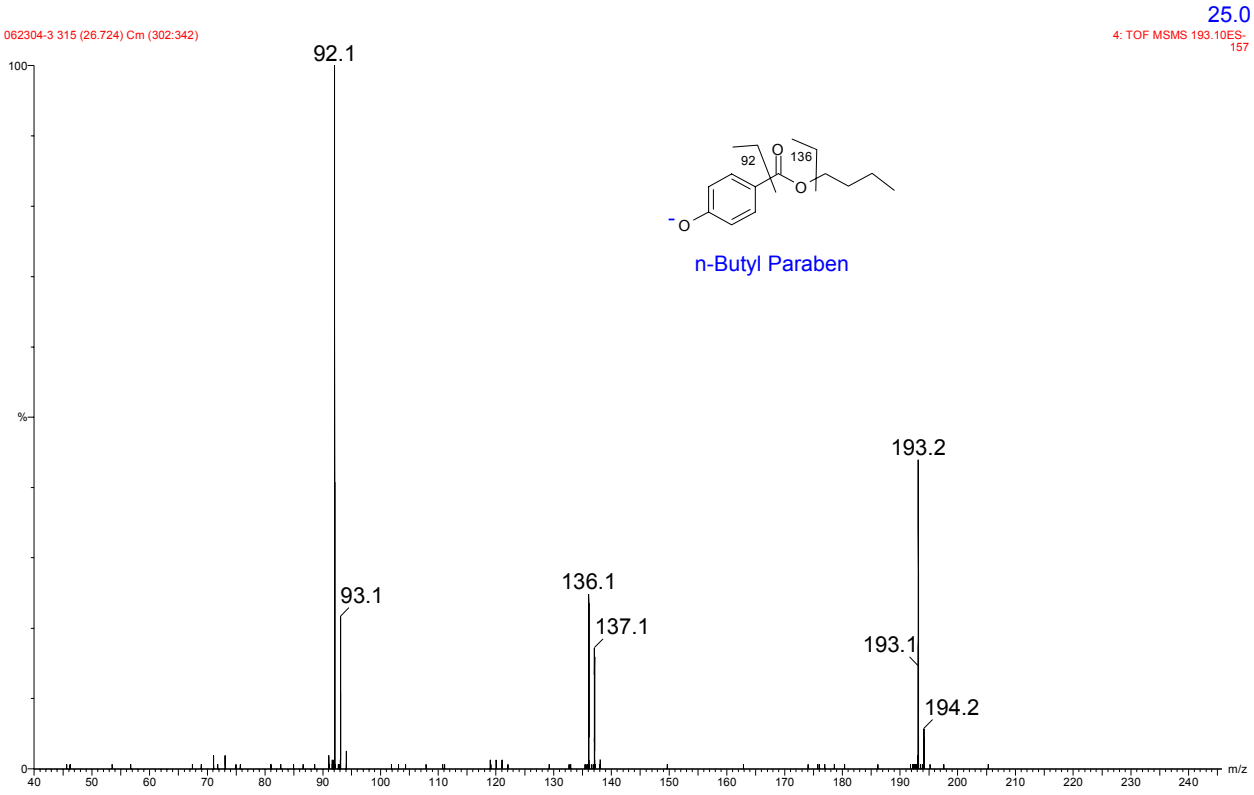


Figure 37: GC/MS single ion chromatogram of molecular ion (*m/z* = 46) of process sample (blank) in dichloromethane (upper panel), and electron impact (EI) mass spectrum of peak eluting at 0.97 minutes (lower panel)

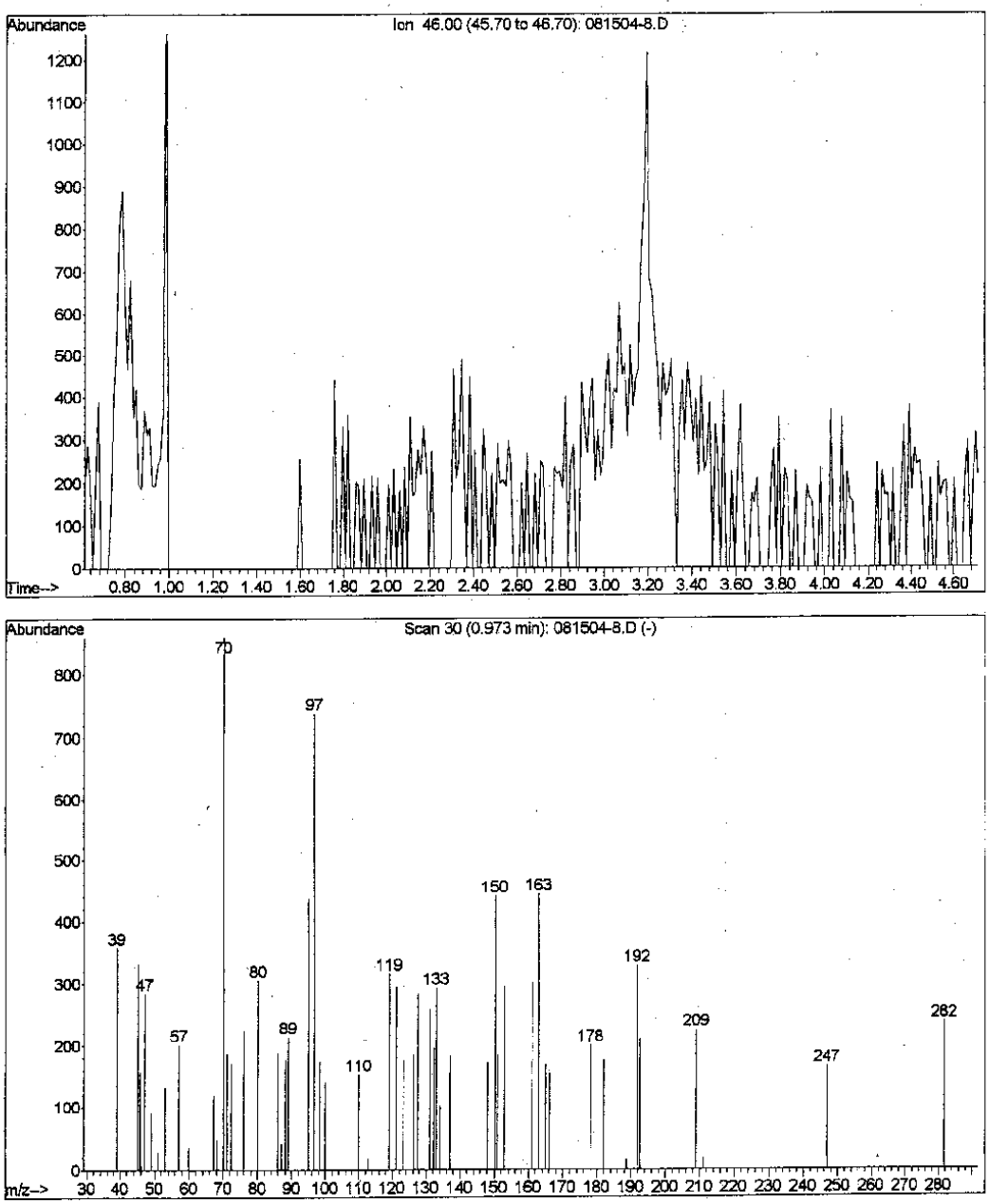


Figure 38: GC/MS single ion chromatogram of molecular ion (*m/z* = 46) of a 3.0% ethanol standard in dichloromethane (upper panel), and electron impact (EI) mass spectrum of ethanol eluting at 0.97 minutes (lower panel)

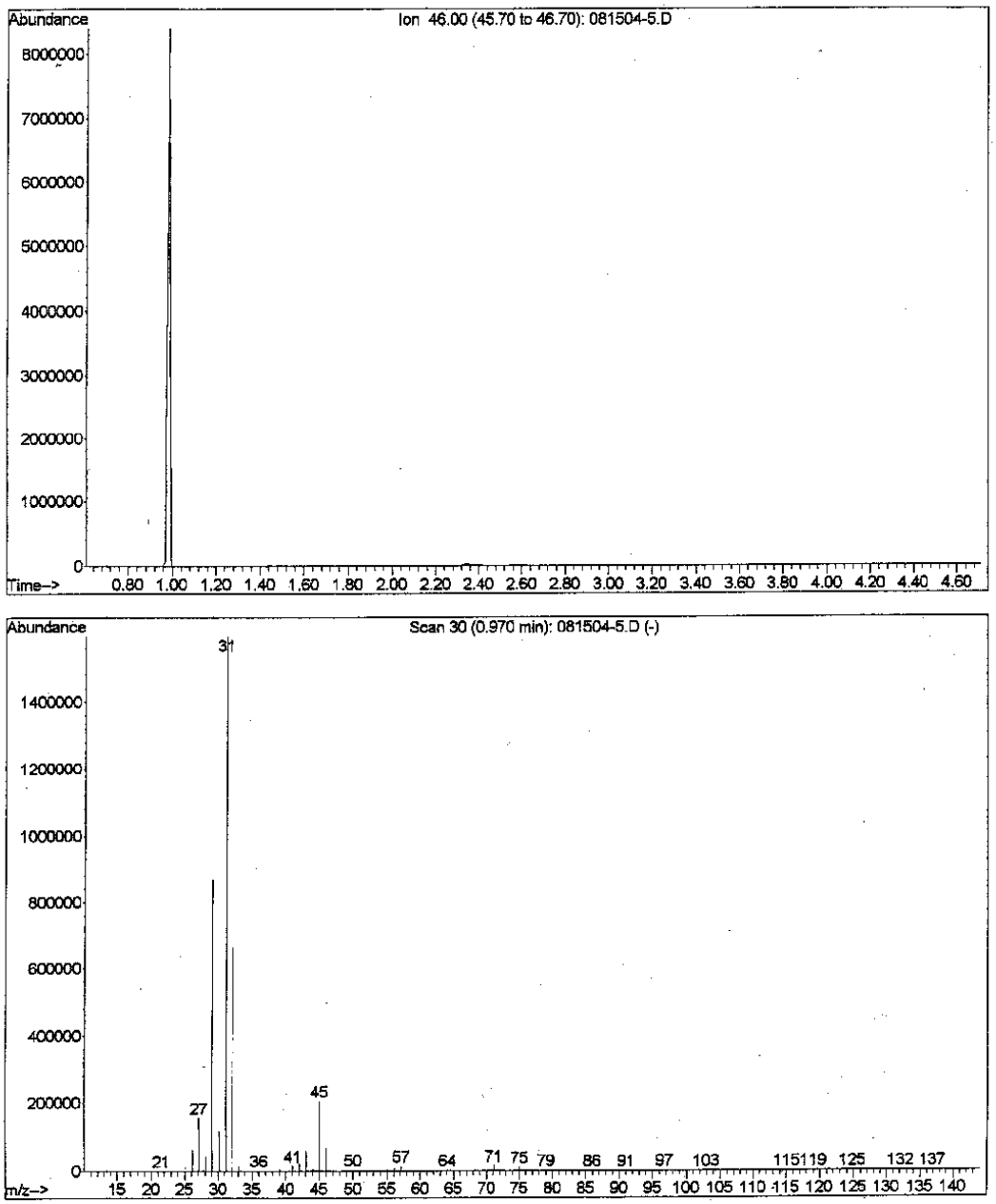


Figure 39: Methylparaben formulation, GC/MS single ion chromatogram of molecular ion (*m/z* = 46) in dichloromethane (upper panel), and electron impact (EI) mass spectrum of peak eluting at 0.97 minutes (lower panel)

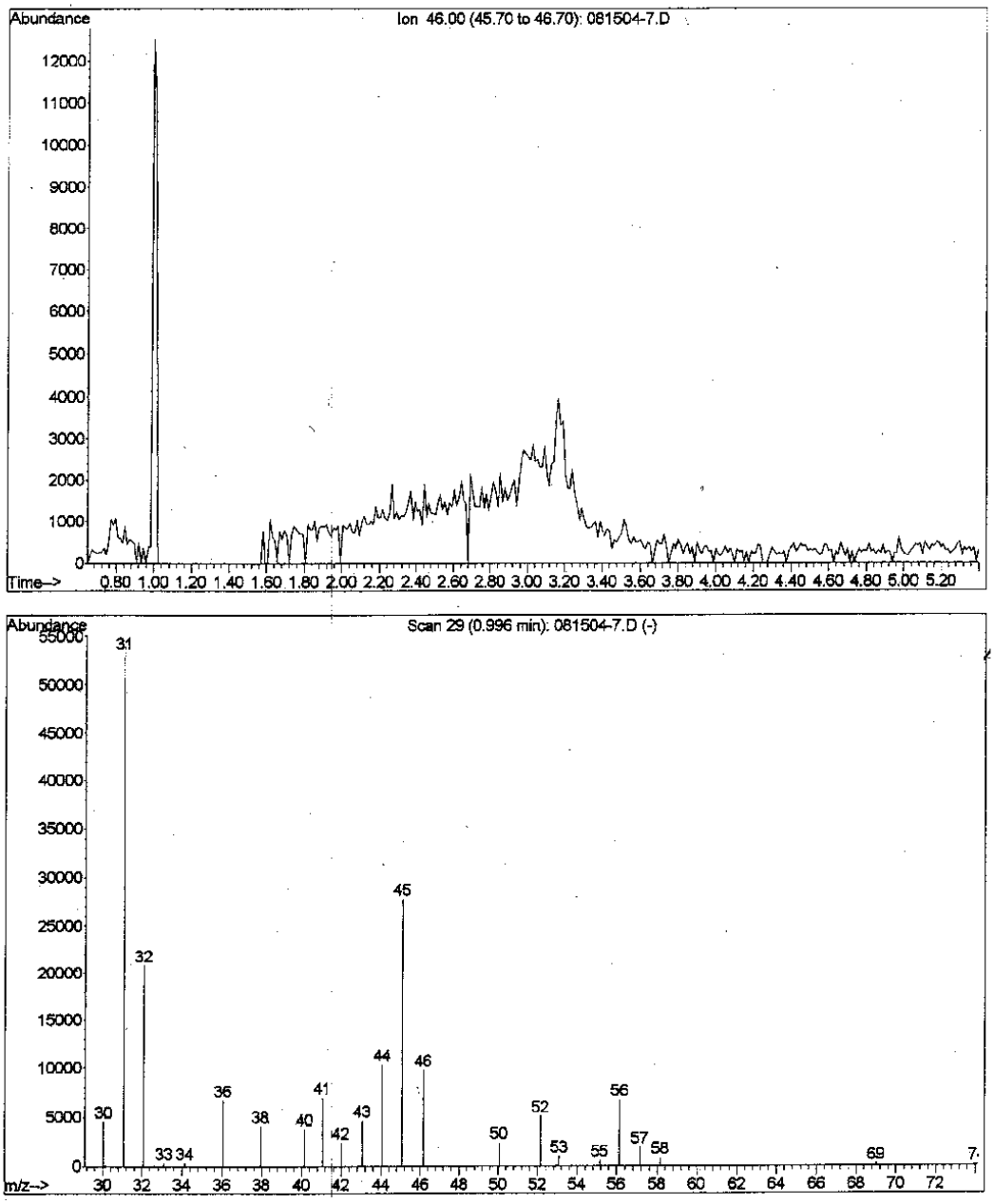


Figure 40: Butylparaben formulation, GC/MS single ion chromatogram of molecular ion (*m/z* = 46) in dichloromethane (upper panel), and electron impact (EI) mass spectrum of peak eluting at 0.97 minutes (lower panel)

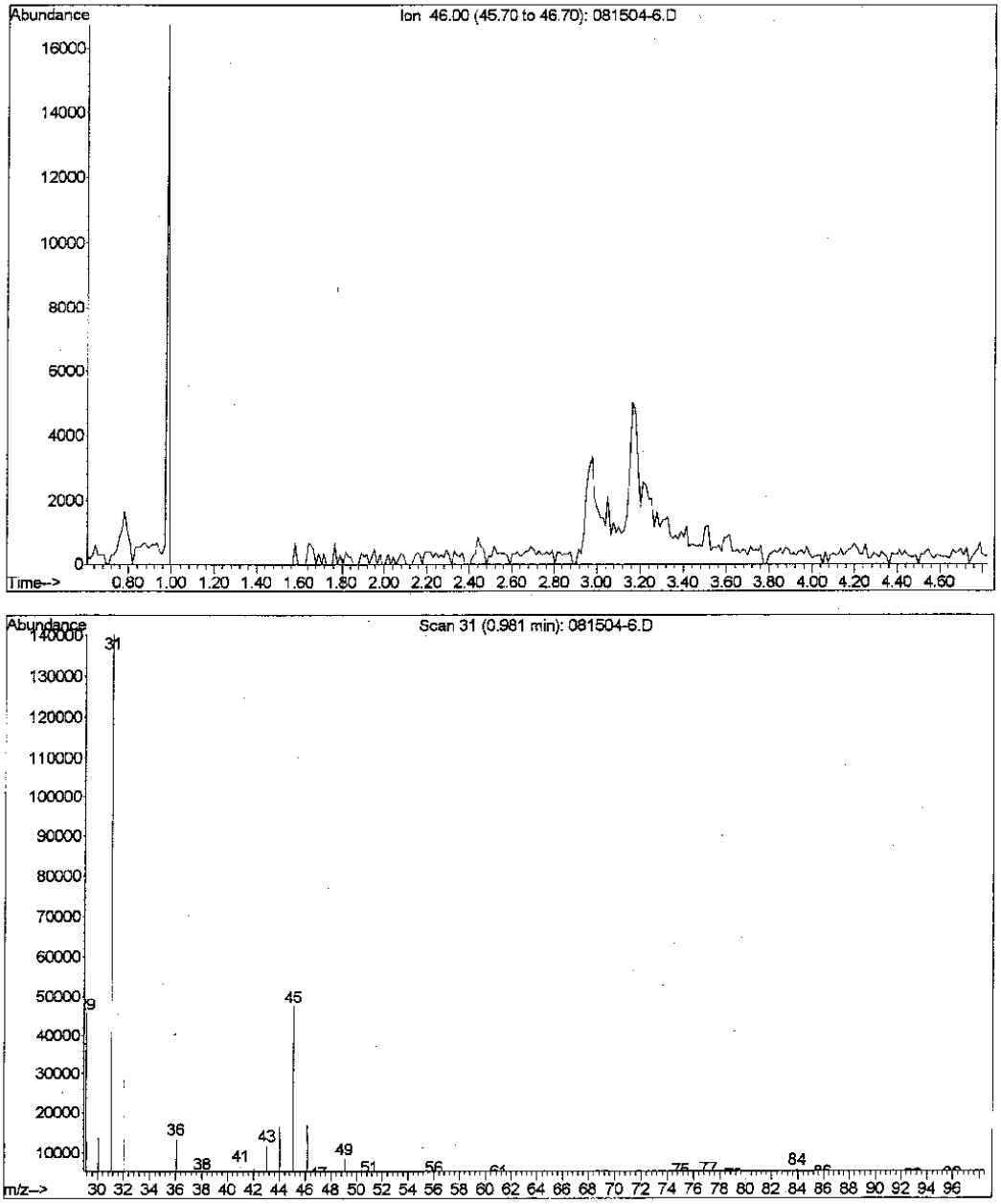
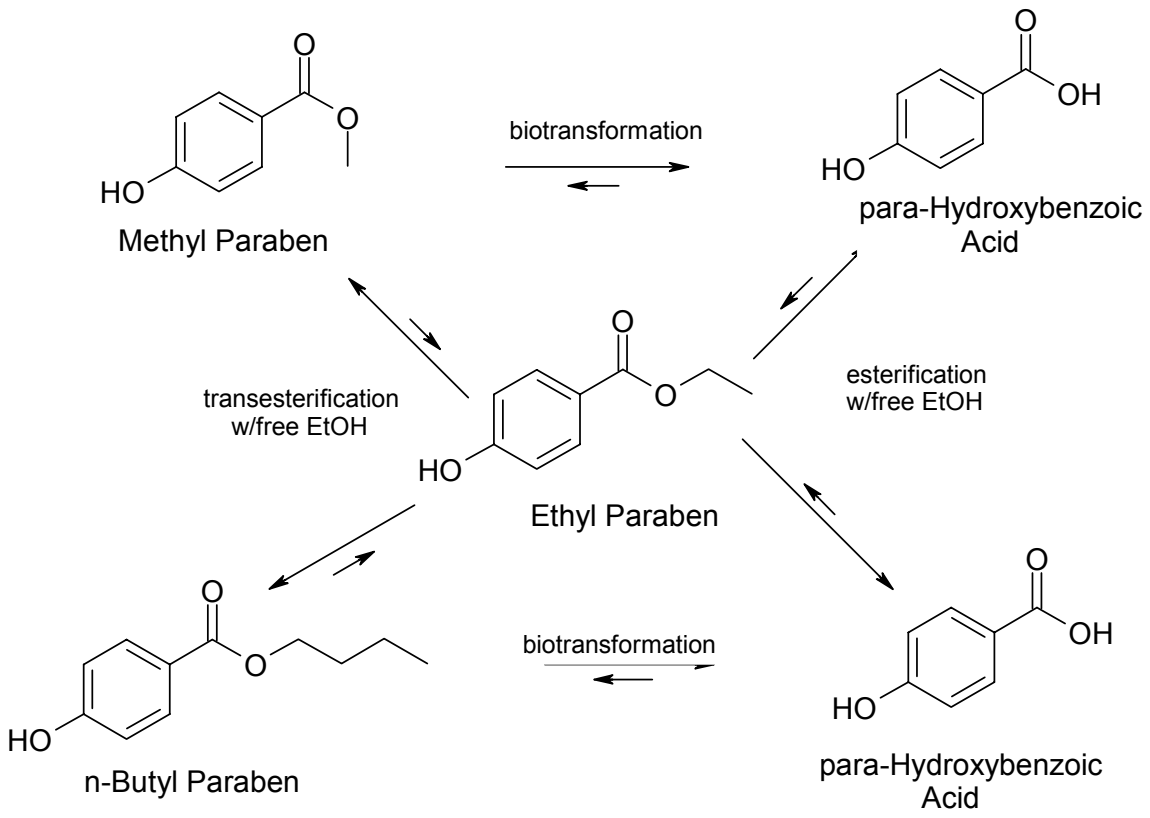


Figure 41: Biotransformation of methylparaben and butylparaben, and formation of ethylparaben



APPENDICES

APPENDICES

EXPLANATORY NOTES

ABBREVIATIONS:

absorbed	receptor fluid + receptor wash
absorbable	absorbed + skin
BP	butylparaben
equiv	equivalent
h	hour(s)
MP	methylparaben
NA	not applicable
SD	standard deviation
unabsorbed	skin wash + donor chamber + tape strips

APPENDIX A

Certificates of Analysis

PROTAMEEN CHEMICALS INC.

375 Minnisink Road
Totowa, N.J. 07511
Office: 973-256-4374
Fax: 973-256-6764

Certificate of Analysis

Product Number: 670
Product Name: METHYL PARABEN NF
Lot Number: M3146

Customer Name:
Customer PO#:
Customer Product Code:
Customer Prod Name:

Test Name:	Range:	Result:
Appearance @ 25dgr	White Powder	White Crystalline
Assay (%)	99.0 - 100.5	99.9
Identification A,B,C	Meets NF standards	Pass
Melting Point Celcius	125 - 128	127
Acidity	Meets NF Requirments	Conforms
Loss on Drying %	0.50 Maximum	0.01
Residue on Ignition	0.05 Maximum	0.01
Color of Solution	Passes	Pass
Chromatographic Purity	Passes	Pass
Organic Volatile Impurities	Meets NF standards	Will Comply

Expiration Date: 4/7/2006
Manufacture Date: 4/8/2003
Issue Date: 9/22/2003
Signature: JOHN J. BRODZINSK TECHNICAL DIRECTC

Remarks: MANUFACTURED BY UENO FINE CHEMICALS

PROTAMEEN CHEMICALS INC.

375 Minnisink Road
Totowa, N.J. 07511
Office: 973-256-4374
Fax: 973-256-6764

Certificate of Analysis

Product Number: 330
Product Name: BUTYL PARABEN
Lot Number: B3140

Customer Name:
Customer PO#:
Customer Product Code:
Customer Prod Name:

Test Name:	Range:	Result:
Appearance @ 25dgr	White crystalline powder	White crystalline powder
Assay (%)	99.0 - 100.5	99.5
Identification - IR	Complies with standard	Pass
Melting Range Celcius	68 - 72	70
Acidity	Complies wiith standard	Conforms
Loss on Drying %	0.5 Maximum	0.02
Residue on Ignition	0.05 Maximum	0.05
Organic Volatile Impurities	Complies with standard	Will Comply

Expiration Date: 2/10/2006 **Manufacture Date:** 2/11/2003

Issue Date: 10/14/2003 **Signature:** JOHN J. BRODZINSK
TECHNICAL DIRECTO

Remarks: MANUFACTURED BY UENO FINE CHEMICALS

Safety data sheet **Date of issue 15 January 2004**

Amersham Biosciences UK Limited Amersham Place Little Chalfont
Buckinghamshire HP7 9NA UK Telephone: +44 (0)870 606 1921

[redacted]

Product name : 4-Hydroxybenzoic acid methyl ester CAS No. 99-76-3 EC No. 202-785-7

R: 36/37/38 Irritating to eyes, respiratory system and skin.
S: 26-36 In case of contact with eyes, rinse immediately with water and seek medical advice. Wear suitable protective clothing.

Composition : 4-Hydroxybenzoic acid methyl ester is supplied as a radioactive solid.

Hazards identification : 4-Hydroxybenzoic acid methyl ester is classified as irritating to eyes, respiratory system and skin.

First aid measures : In case of contact, immediately flush eyes and skin with water. If inhaled, remove to fresh air. If swallowed, wash out mouth with water. In severe cases, or in case of contact with eyes, seek medical attention.

Fire fighting measures : For small fires only. Wear protective clothing. Use water spray, carbon dioxide, dry chemical powder or appropriate foam.

Accidental release : Avoid exposure to dust. Wear protective clothing including laboratory overalls, safety glasses and gloves. Treat as for spills of radioactive material (see Handling Instructions for Radioactive materials).

Handling and storage : Follow handling instructions for Radioactive materials. Wear protective clothing including laboratory overalls, safety glasses and gloves. Use in a chemical fume hood. Do not breathe dust. Avoid contact with skin and eyes. Avoid prolonged or repeated exposure. Keep container tightly closed. Wash thoroughly after handling.

Personal protection: See above instructions for handling and storage.

Physical and chemical properties : Form and appearance : solid
Melting point : 125°C

Stability and reactivity : 4-Hydroxybenzoic acid methyl ester is stable.
Avoid strong oxidising agents and strong bases.

Toxicological information : LD_{50}: 8000mg/kg oral, mouse.
Causes skin and eye irritation. May be harmful if absorbed through the skin, inhaled or swallowed. Material is irritating to mucous membranes and upper respiratory tract.

Ecological information : Not available.

Disposal considerations : Dispose of waste material as for radioactive waste.
(See: Instructions relating to the Handling and Disposal of Radioactive Materials.)

Transport information : As applicable to radioactive materials.

Regulatory information : The information contained in this Safety Data Sheet is based on published sources of information and is believed to be correct. It should be used a guide only. It is the responsibility of the user of this product to carry out an assessment of workplace risks, as may be required under national legislation.

CAUTION - RADIOACTIVE MATERIAL

Product Specification

http://www.customlabelling.com
Amersham Biosciences UK Limited
Amersham Place Little Chalfont Buckinghamshire HP7 9NA UK
Telephone +44 (0)870 606 1921

Certificate number LRQ 0925289

Before using this product, please read the instructions overleaf for safe handling, storage and disposal

4-Hydroxy[ring-U-^{14}C]benzoic acid methyl ester

Code CFQ13765 Batch 1 [redacted]

Pack size 185 MBq, 5mCi

Technical data

Specific activity			
determined by mass spectrometry	:	555 MBq/mmol,	15 mCi/mmol
determined by gravimetric analysis	:	3.59 MBq/mg	97 µCi/mg
Molecular weight	:	152.6 (at this specific activity)	
Radiochemical purity			
by high performance liquid chromatography	:	99.5%	

Column	:	Inertsil ODS 3V 5µm (250 x 4.6mm)					
Solvent A	:	0.1% trifluoroacetic acid in water					
Solvent B	:	0.1% trifluoroacetic acid in acetonitrile					
Gradient	:	Time (mins)	0	15	30	31	35
		% B	0	100	100	0	0
Flow rate	:	1 ml/min					
UV detection	:	280 nm					

Analysed on 28th January 2004

Chemical identity

The material co-chromatographs with commercially available material in the above chromatographic system.

The mass spectrum is consistent with the proposed structure and a non-labelled reference.

The ^{1}H-nmr spectrum is consistent with the proposed structure and a non-labelled reference.

Amersham Biosciences

Safety data sheet **Date of issue 15 January 2004**

Amersham Biosciences UK Limited Amersham Place Little Chalfont
Buckinghamshire HP7 9NA UK Telephone: +44 (0)870 606 1921

[redacted]

Product name : 4-Hydroxybenzoic acid butyl ester CAS No. 94-26-8 EC No. 202-318-7

S: 22-24/25 Do not breathe dust. Avoid contact with skin and eyes.

Composition : 4-Hydroxybenzoic acid butyl ester is supplied as a radioactive solid.

Hazards identification : 4-Hydroxybenzoic acid butyl ester is not classified as hazardous.

First aid measures : In case of contact, immediately flush eyes and skin with water. If inhaled, remove to fresh air. If swallowed, wash out mouth with water. In severe cases, or in case of contact with eyes, seek medical attention.

Fire fighting measures : For small fires only. Wear protective clothing. Use water spray, carbon dioxide, dry chemical powder or appropriate foam.

Accidental release : Avoid exposure to dust. Wear protective clothing including laboratory overalls, safety glasses and gloves. Treat as for spills of radioactive material (see Handling Instructions for Radioactive materials).

Handling and storage : Follow handling instructions for Radioactive materials. Wear protective clothing including laboratory overalls, safety glasses and gloves. Use in a chemical fume hood. Do not breathe dust. Avoid contact with skin and eyes. Avoid prolonged or repeated exposure. Keep container tightly closed. Wash thoroughly after handling.

Personal protection: See above instructions for handling and storage.

Physical and chemical properties : Form and appearance : solid
Melting point : 68°C
Boiling point : 156-157°C (3.5 mmHg)

Stability and reactivity : 4-Hydroxybenzoic acid butyl ester is stable.
Avoid strong oxidising agents.

Toxicological information : LD_{50}: 13200mg/kg oral, mouse.
May cause skin and eye irritation. May be harmful if absorbed through the skin, inhaled or swallowed. Material may be irritating to mucous membranes and upper respiratory tract.

Ecological Information : Not available.

Disposal considerations : Dispose of waste material as for radioactive waste.
(See: Instructions relating to the Handling and Disposal of Radioactive Materials.)

Transport information : As applicable to radioactive materials.

Regulatory information : The information contained in this Safety Data Sheet is based on published sources of information and is believed to be correct. It should be used a guide only. It is the responsibility of the user of this product to carry out an assessment of workplace risks, as may be required under national legislation.

CAUTION - RADIOACTIVE MATERIAL

Product Specification

http://www.customlabelling.com
Amersham Biosciences UK Limited
Amersham Place Little Chalfont Buckinghamshire HP7 9NA UK
Telephone +44 (0)870 606 1921

Certificate number LRQ 0925285

Before using this product, please read the instructions overleaf for safe handling, storage and disposal

4-Hydroxy[ring-U-^{14}C]benzoic acid butyl ester

Code CFQ13766 Batch 1 [redacted]

Pack size 185 MBq, 5mCi

Technical data

Specific activity			
determined by mass spectrometry	:	518 MBq/mmol,	14 mCi/mmol
determined by gravimetric analysis	:	2.78 MBq/mg	75 µCi/mg
Molecular weight	:	194.7 (at this specific activity)	
Radiochemical purity			
by high performance liquid chromatography	:	99.2%	

Column	:	Inertsil ODS 3V 5µm (250 x 4.6mm)					
Solvent A	:	0.1% trifluoroacetic acid in water					
Solvent B	:	0.1% trifluoroacetic acid in acetonitrile					
Gradient	:	Time (mins)	0	15	30	31	35
		% B	0	100	100	0	0
Flow rate	:	1 ml/min					
UV detection	:	254 nm					

Analysed on 29th January 2004

Chemical identity

The material co-chromatographs with commercially available material in the above chromatographic system.

The mass spectrum is consistent with the proposed structure and a non-labelled reference.

The ^{1}H-nmr spectrum is consistent with the proposed structure and a non-labelled reference.

Amersham Biosciences

Cosmetech
LABORATORIES, INC.
39 PLYMOUTH STREET • SUITE 4 • FAIRFIELD, NEW JERSEY 07004-1681
TELEPHONE (973) 882-5151 • FAX (973) 882-1222
E-MAIL Ken@Cosmetech.com E-MAIL Irwin@Cosmetech.com

KENNETH KLEIN
PRESIDENT

IRWIN PALEFSKY
SR. VICE PRESIDENT

March 1, 2004

Mr. Bill Fasano
Stine Haskell Research Center
1090 Elkton Road
Building Haskell 1 Room 620
Newark, DE 19714

Dear Mr. Fasano:

Enclosed are the phases necessary to make the Oil in Water emulsion formulation that you will use for the incorporation of parabens.

Please let us know if you have any questions.

Best regards,

COSMETECH LABORATORIES, INC.

Irwin Palefsky
Sr. Vice President

IP/fh
Encl.

COSMETECH LABORATORIES, INC.

PRODUCT: PREMIX EMULSION FOR PARABEN STUDY
PURPOSE: TO PREPARE AN OIL-IN-WATER EMULSION FOR PARABEN INCORPORATION
PREPARED FOR: CTFA LAB #CLI: 1662701 DATE: 03/01/04
PREPARED BY: NW SUPERSEDES: DATE:
APPROVED BY: K.KLEIN

NO.	PHASE	INGREDIENT (TRADE NAME)	INCI DESIGNATION	SUPPLIER	% BY WEIGHT	BATCH SIZE
						1500.00
1	A	WATER	WATER (AQUA)		76.00	1140.00
2	A	GLYCERIN 96%	GLYCERIN	COGNIS	3.00	45.00
3	A	CARBOPOL 980 (2% SOLN)	CARBOMER	NOVEON	7.50	112.50
4	B	STEARIC ACID XXX	STEARIC ACID	COGNIS	4.00	60.00
5	B	CETYL ALCOHOL	CETYL ALCOHOL	COGNIS	0.25	3.75
6	B	STEARYL ALCOHOL	STEARYL ALCOHOL	COGNIS	0.25	3.75
7	B	DRAKEOL #7	#N/A	#N/A	5.00	75.00
8	B	CERASYNT Q	GLYCERYL STEARATE SE	ISP	2.00	30.00
9	C	TRIETHANOLAMINE 99%	TRIETHANOLAMINE	UNION CARBIDE	1.20	18.00
					—	—
		TOTAL			99.20	1488.00

MANUFACTURING INSTRUCTIONS:
FOR FINAL FORMULA PREPARATION HEAT PHASE A TO 75C. ADD PARABENS TO PHASE A.HEAT PHASE B TO 75C.
ADD PHASE B TO PHASE A. ADD PHASE C TO PHASE A/B. COOL WITH SLOW MIXING UNTIL
TEMPERATURE REACHES 35C. PACKAGE AT 35C.

LAB CONTROL:
pH: 6.50-7.25
OTHER INFORMATION:

VISCOSITY (CPS): 12,500 CPS- 25,000 CPS

H# 22703-288

SIGMA-ALDRICH

3050 Spruce Street
Saint Louis, Missouri 63105 USA
Telephone (800) 521-8956 • (314) 771-5765
Fax (800) 325-5052 • (314) 77-5757
Visit Us At www.sigma-aldrich.com

Certificate of Analysis

DUPONT
JULIA WANG, H1/1313
DUPONT - STINE-HASKELL/B1
ELKTON RD
NEWARK DE 19711

PO NBR: PC294063

PRODUCT NUMBER: W271004-SPEC LOT NUMBER: 14312HB

PRODUCT NAME: METHYL P-HYDROXYBENZOATE, 99+%, FCC

FORMULA: C8H8O3 FORMULA WEIGHT: 152.15

APPEARANCE	WHITE POWDER
MELTING POINT	125.4-127.4 DEGREES CELSIUS
INFRARED SPECTRUM	CONFORMS TO STRUCTURE.
TITRATION	99.8%* (WITH HCL) (DRIED BASES)
GAS LIQUID CHROMATOGRAPHY	99.9 %
LOSS ON DRYING	0.02% (5 HOURS, ROOM TEMPERATURE)
RESIDUE ON IGNITION	0.01% (15 MINUTES, 800 DEGREES C)
SOLUBILITY	1G/10ML, 95% ETOH, CLEAR,COLORLESS SOLUTION
ACIDITY	PASSES TEST
HEAVY METALS	<10 PPM
	* SUPPLIER DATA
	MEETS FCC-IV, SUPPLEMENT III SPECIFICATION
QUALITY CONTROL ACCEPTANCE DATE	JUNE, 2003

ALDRICH CHEMICAL COMPANY
RONNIE MARTIN
DECEMBER 11, 2003

*We are Committed to the success of our Customers, Employees and Shareholders through leadership in **Life Science, High Technology** and Service.*

H# 22703-289

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Certificate of Analysis

DUPONT
JULIA WANG, H1/1313
DUPONT - STINE-HASKELL/B1
ELKTON RD
NEWARK DE 19711

PO NBR: PC294063

PRODUCT NUMBER: W220302-SPEC LOT NUMBER: 12116CU

PRODUCT NAME: BUTYL P-HYDROXYBENZOATE, 99+%

FORMULA: C11H14O3 FORMULA WEIGHT: 194.23

APPEARANCE	WHITE CRYSTALLINE POWDER
MELTING POINT	69.1-70.3 DEGREES CELSIUS
INFRARED SPECTRUM	CONFORMS TO STRUCTURE AND STANDARD AS ILLUSTRATED ON PAGE 620C OF EDITION I, VOLUME 1 OF "THE ALDRICH LIBRARY OF FT-IR SPECTRA".
GAS LIQUID CHROMATOGRAPHY	99.9 %
SOLUBILITY	5% IN 95% ETHANOL; CLEAR, COLORLESS SOLUTION
QUALITY CONTROL ACCEPTANCE DATE	MARCH 1999

ALDRICH CHEMICAL COMPANY
RONNIE MARTIN
DECEMBER 11, 2003

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H# 22703-290

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Saint Louis, Missouri 63105 USA
Telephone (800) 521-8956 • (314) 771-5765
Fax (800) 325-5052 • (314) 77-5757
Visit Us At www.sigma-aldrich.com

Certificate of Analysis

PO NBR:

PRODUCT NUMBER: W39860-8 LOT NUMBER: 26026BB

PRODUCT NAME: P-HYDROXYBENZOIC ACID, 99+%

FORMULA: C7H6O3 FORMULA WEIGHT: 138.12

APPEARANCE	WHITE POWDER
MELTING POINT	215.5-217.3 DEGREES CELSIUS
INFRARED SPECTRUM	CONFORMS TO STRUCTURE.
TITRATION	100.8 % (WITH NAOH)
GAS LIQUID CHROMATOGRAPHY	99.9 %
SOLUBILITY	50MG/ML, MEOH, VERY SLIGHTLY HAZY,COLORLESS SOLUTION
QUALITY CONTROL ACCEPTANCE DATE	MARCH, 2003

ALDRICH CHEMICAL COMPANY
RONNIE MARTIN
DECEMBER 22, 2003

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APPENDIX B

Methylparaben – Rat

Application amounts and rates

Skin Replicate	Activity applied (μCi)	Total MP (μg)	MP per area (μg/cm^2)
1	0.81	41.0	64.0
2	0.80	40.7	63.7
3	0.80	40.4	63.1
4	0.80	40.8	63.7
5	0.79	40.1	62.6
6	0.81	41.0	64.0
7	0.80	40.5	63.3
8	0.80	40.6	63.4
9	0.79	40.3	63.0
10	0.80	40.7	63.6
Mean	0.80	40.61	63.45
SD	0.01	0.29	0.45

Cumulative amount of radioactivity absorbed per area (µg equiv/cm^2)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	2.97	9.12	13.28	16.11	18.19	19.75	22.41	24.37	26.14	27.78	29.29	30.66	31.92	33.05	34.07
3 (2)	8.42	19.73	25.25	27.54	28.70	29.57	31.00	32.17	33.30	34.37	35.34	36.22	37.04	37.78	38.47
4 (3)	6.18	15.97	19.52	20.92	21.80	22.47	23.68	24.79	25.79	26.81	27.72	28.55	29.28	29.95	30.51
5 (4)	2.57	10.00	14.43	17.11	19.15	20.78	23.52	25.74	27.95	29.95	31.75	33.35	34.76	35.84	36.89
6 (5)	3.10	7.19	9.36	10.99	12.48	13.90	16.96	20.02	23.03	26.04	28.86	31.47	33.88	36.10	38.09
8 (6)	1.24	6.64	12.29	15.98	18.54	20.36	23.03	24.86	26.40	27.77	29.02	30.17	31.22	32.20	33.10
10 (7)	2.47	9.48	13.99	16.70	18.64	20.13	23.07	25.68	28.09	30.35	32.29	34.08	35.68	37.16	38.52
11 (8)	1.41	5.47	7.91	9.63	11.11	12.52	15.57	18.70	21.87	25.11	28.11	30.92	33.45	35.91	37.97
12 (9)	0.65	2.91	5.01	6.84	8.57	10.06	13.03	15.92	18.70	21.40	24.01	26.43	28.71	30.93	32.91
13 (10)	0.65	3.90	6.09	7.50	8.63	9.60	11.69	13.82	16.02	18.26	20.40	22.46	24.44	26.36	28.01
MEAN	2.97	9.04	12.71	14.93	16.58	17.91	20.40	22.61	24.73	26.79	28.68	30.43	32.04	33.53	34.85
SD	2.51	5.26	6.20	6.41	6.37	6.28	5.93	5.45	4.99	4.56	4.20	3.94	3.77	3.68	3.71

Concentration-time course of total radioactivity (µg equiv/mL)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	1.20	2.49	1.69	1.14	0.84	0.63	0.54	0.40	0.36	0.33	0.30	0.28	0.25	0.23	0.21
3 (2)	3.41	4.58	2.23	0.93	0.47	0.35	0.29	0.24	0.23	0.22	0.20	0.18	0.17	0.15	0.14
4 (3)	2.50	3.97	1.44	0.57	0.36	0.27	0.24	0.22	0.20	0.21	0.18	0.17	0.15	0.13	0.11
5 (4)	1.04	3.01	1.79	1.09	0.83	0.66	0.55	0.45	0.44	0.41	0.36	0.32	0.29	0.22	0.21
6 (5)	1.26	1.66	0.88	0.66	0.61	0.58	0.62	0.62	0.61	0.61	0.57	0.53	0.49	0.45	0.40
8 (6)	0.50	2.19	2.29	1.49	1.04	0.74	0.54	0.37	0.31	0.28	0.25	0.23	0.21	0.20	0.18
10 (7)	1.00	2.84	1.83	1.10	0.78	0.61	0.59	0.53	0.49	0.46	0.39	0.36	0.32	0.30	0.27
11 (8)	0.57	1.65	0.99	0.70	0.60	0.57	0.61	0.63	0.64	0.66	0.60	0.57	0.51	0.50	0.42
12 (9)	0.26	0.92	0.85	0.74	0.70	0.60	0.60	0.58	0.56	0.55	0.53	0.49	0.46	0.45	0.40
13 (10)	0.27	1.32	0.88	0.57	0.46	0.39	0.42	0.43	0.44	0.45	0.43	0.42	0.40	0.39	0.33
MEAN	1.20	2.46	1.49	0.90	0.67	0.54	0.50	0.45	0.43	0.42	0.38	0.35	0.32	0.30	0.27
SD	1.02	1.17	0.56	0.30	0.21	0.15	0.14	0.15	0.15	0.16	0.15	0.14	0.13	0.13	0.11

Cumulative percent of radioactivity absorbed

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	4.64	14.24	20.75	25.17	28.42	30.85	35.01	38.08	40.83	43.41	45.77	47.90	49.87	51.64	53.23
3 (2)	13.22	31.00	39.66	43.25	45.08	46.44	48.70	50.53	52.30	53.98	55.51	56.89	58.18	59.34	60.42
4 (3)	9.79	25.30	30.92	33.14	34.53	35.59	37.50	39.26	40.85	42.47	43.91	45.21	46.38	47.43	48.33
5 (4)	4.04	15.68	22.63	26.84	30.04	32.59	36.89	40.38	43.84	46.99	49.81	52.31	54.53	56.22	57.86
6 (5)	4.96	11.49	14.95	17.55	19.94	22.21	27.09	31.97	36.78	41.59	46.10	50.27	54.11	57.65	60.84
8 (6)	1.94	10.37	19.19	24.95	28.96	31.80	35.95	38.82	41.22	43.37	45.31	47.10	48.75	50.28	51.68
10 (7)	3.90	14.97	22.10	26.37	29.43	31.80	36.44	40.55	44.35	47.93	50.99	53.82	56.35	58.68	60.83
11 (8)	2.22	8.63	12.47	15.18	17.51	19.74	24.55	29.49	34.48	39.60	44.32	48.76	52.74	56.62	59.88
12 (9)	1.03	4.62	7.95	10.85	13.61	15.96	20.68	25.27	29.69	33.97	38.11	41.96	45.57	49.09	52.24
13 (10)	1.03	6.14	9.57	11.79	13.57	15.09	18.38	21.73	25.19	28.72	32.07	35.31	38.43	41.44	44.05
MEAN	4.68	14.24	20.02	23.51	26.11	28.21	32.12	35.61	38.95	42.20	45.19	47.95	50.49	52.84	54.94
SD	3.95	8.29	9.74	10.05	9.98	9.82	9.25	8.50	7.77	7.10	6.56	6.18	5.94	5.84	5.92

Material Balance

Cell ID (replicate)	Receptor Fluid	Receptor wash	Skin Wash	Donor Chamber	Tape Strips	Skin	Total Recovery
1 (1)	53.23	0.26	15.62	0.03	5.57	9.74	84.44
3 (2)	60.42	0.16	13.80	0.02	6.63	3.43	84.46
4 (3)	48.33	0.20	22.31	0.03	4.57	13.52	88.96
5 (4)	57.86	0.39	16.93	0.03	6.85	11.35	93.41
6 (5)	60.84	0.59	17.45	0.02	6.09	13.38	98.37
8 (6)	51.68	0.29	21.90	0.02	6.20	9.20	89.29
10 (7)	60.83	0.47	15.60	0.05	6.26	13.45	96.67
11 (8)	59.88	0.65	16.23	0.03	6.01	15.60	98.40
12 (9)	52.24	0.67	20.06	0.02	3.12	8.10	84.21
13 (10)	44.05	0.66	18.21	0.03	5.18	24.57	92.70
MEAN	54.94	0.43	17.81	0.03	5.65	12.23	91.09
SD	5.92	0.20	2.82	0.01	1.12	5.57	5.66

Cell ID (replicate)	Absorbed	Absorbable	Unabsorbed
1 (1)	53.49	63.22	21.22
3 (2)	60.58	64.01	20.45
4 (3)	48.53	62.04	26.92
5 (4)	58.25	69.60	23.81
6 (5)	61.43	74.81	23.56
8 (6)	51.98	61.17	28.12
10 (7)	61.30	74.75	21.91
11 (8)	60.53	76.14	22.26
12 (9)	52.91	61.01	23.19
13 (10)	44.71	69.28	23.42
MEAN	55.37	67.61	23.49
SD	5.92	6.06	2.40

Cumulative amount of methylparaben absorbed per area (μg/cm^2)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	0.51	1.38	2.80	3.58	3.85	4.30	5.06	5.69	5.93	6.45	6.87	7.27	7.44	7.82	8.10
3 (2)	2.55	6.26	10.83	12.20	12.54	13.05	13.87	14.54	14.88	15.46	16.02	16.49	16.80	17.19	17.55
4 (3)	1.43	2.20	3.14	3.40	3.49	3.65	3.97	4.28	4.39	4.62	4.82	5.00	5.08	5.23	5.35
5 (4)	0.23	0.72	0.93	1.04	1.08	1.16	1.30	1.44	1.51	1.61	1.73	1.85	1.90	1.96	2.02
6 (5)	0.96	2.58	3.26	3.81	4.11	4.62	5.77	6.92	7.60	8.66	9.62	10.51	10.92	11.61	12.25
8 (6)	0.29	1.93	2.38	2.53	2.65	2.69	2.82	3.02	3.24	3.57	3.87	4.15	4.31	4.53	4.72
10 (7)	0.57	4.17	4.97	5.32	5.57	5.77	6.03	6.33	7.17	8.19	9.09	9.87	10.36	11.00	11.57
11 (8)	0.60	1.01	1.61	2.04	2.35	2.78	3.66	4.51	5.16	5.99	6.76	7.45	7.89	8.47	9.00
12 (9)	0.38	1.21	1.60	1.93	2.20	2.54	3.35	4.21	4.91	5.64	6.36	6.99	7.45	8.02	8.52
13 (10)	0.22	0.94	1.20	1.36	1.45	1.58	1.88	2.18	2.40	2.72	2.99	3.25	3.45	3.72	3.96
MEAN	0.77	2.24	3.27	3.72	3.93	4.21	4.77	5.31	5.72	6.29	6.81	7.28	7.56	7.96	8.30
SD	0.73	1.74	2.91	3.25	3.31	3.41	3.55	3.68	3.76	3.93	4.10	4.25	4.35	4.49	4.62

Concentration-time course of methylparaben (µg equiv/mL)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	0.21	0.35	0.58	0.32	0.11	0.18	0.15	0.13	0.05	0.10	0.09	0.08	0.03	0.08	0.06
3 (2)	1.03	1.50	1.85	0.56	0.14	0.21	0.17	0.14	0.07	0.12	0.11	0.09	0.06	0.08	0.07
4 (3)	0.58	0.31	0.38	0.11	0.03	0.07	0.06	0.06	0.02	0.05	0.04	0.04	0.02	0.03	0.02
5 (4)	0.09	0.20	0.08	0.05	0.02	0.03	0.03	0.03	0.01	0.02	0.02	0.03	0.01	0.01	0.01
6 (5)	0.39	0.65	0.28	0.22	0.12	0.21	0.23	0.23	0.14	0.21	0.19	0.18	0.08	0.14	0.13
8 (6)	0.12	0.66	0.18	0.06	0.05	0.02	0.03	0.04	0.05	0.07	0.06	0.05	0.03	0.04	0.04
10 (7)	0.23	1.46	0.33	0.14	0.10	0.08	0.05	0.06	0.17	0.21	0.18	0.16	0.10	0.13	0.12
11 (8)	0.24	0.17	0.24	0.17	0.13	0.17	0.18	0.17	0.13	0.17	0.16	0.14	0.09	0.12	0.11
12 (9)	0.16	0.33	0.16	0.13	0.11	0.14	0.16	0.17	0.14	0.15	0.15	0.13	0.09	0.12	0.10
13 (10)	0.09	0.29	0.11	0.07	0.04	0.05	0.06	0.06	0.04	0.06	0.05	0.05	0.04	0.06	0.05
MEAN	0.31	0.59	0.42	0.18	0.08	0.12	0.11	0.11	0.08	0.12	0.11	0.09	0.06	0.08	0.07
SD	0.29	0.50	0.52	0.15	0.05	0.07	0.07	0.07	0.06	0.07	0.06	0.05	0.03	0.04	0.04

Cumulative percent of methylparaben absorbed

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	0.80	2.15	4.37	5.59	6.02	6.73	7.91	8.90	9.26	10.07	10.74	11.36	11.62	12.22	12.65
3 (2)	4.00	9.83	17.01	19.16	19.69	20.50	21.78	22.84	23.38	24.29	25.16	25.89	26.39	27.00	27.56
4 (3)	2.26	3.48	4.97	5.39	5.52	5.78	6.29	6.78	6.95	7.31	7.64	7.92	8.05	8.29	8.48
5 (4)	0.36	1.13	1.45	1.63	1.69	1.81	2.03	2.25	2.36	2.53	2.71	2.91	2.97	3.08	3.17
6 (5)	1.54	4.11	5.21	6.08	6.57	7.39	9.22	11.06	12.15	13.83	15.36	16.78	17.45	18.55	19.57
8 (6)	0.46	3.01	3.71	3.95	4.14	4.20	4.40	4.71	5.06	5.58	6.05	6.47	6.73	7.07	7.38
10 (7)	0.90	6.58	7.85	8.40	8.79	9.11	9.52	9.99	11.33	12.94	14.36	15.59	16.36	17.36	18.27
11 (8)	0.95	1.60	2.54	3.21	3.70	4.38	5.78	7.12	8.13	9.44	10.66	11.75	12.44	13.35	14.19
12 (9)	0.61	1.92	2.55	3.06	3.49	4.03	5.32	6.68	7.80	8.95	10.09	11.10	11.82	12.74	13.52
13 (10)	0.35	1.48	1.89	2.14	2.28	2.48	2.95	3.43	3.78	4.27	4.70	5.11	5.42	5.85	6.23
MEAN	1.22	3.53	5.16	5.86	6.19	6.64	7.52	8.38	9.02	9.92	10.75	11.49	11.92	12.55	13.10
SD	1.14	2.75	4.58	5.10	5.19	5.35	5.58	5.79	5.92	6.18	6.46	6.71	6.86	7.08	7.30

Cumulative amount of 4-hydroxybenzoic acid absorbed per area (µg/cm^2)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	1.23	6.93	10.57	12.81	14.41	15.55	17.40	18.74	20.17	21.48	22.55	23.64	24.50	25.45	26.22
3 (2)	3.67	10.65	12.72	13.56	13.97	14.29	14.89	15.40	15.82	16.31	16.75	17.20	17.52	17.87	18.25
4 (3)	2.58	7.60	10.08	10.64	10.93	11.17	11.63	12.11	12.46	12.91	13.28	13.66	13.90	14.20	14.46
5 (4)	0.84	14.33	17.76	19.58	20.53	21.43	22.96	24.26	25.17	26.34	27.36	28.27	28.78	29.39	30.00
6 (5)	0.28	1.29	1.93	2.40	2.69	3.08	3.93	4.86	5.40	6.44	7.40	8.32	8.86	9.61	10.22
8 (6)	0.06	1.33	3.60	5.42	5.80	6.62	7.70	9.15	9.60	10.46	11.23	11.97	12.29	12.87	13.44
10 (7)	0.15	2.39	3.48	4.22	4.38	4.76	5.49	6.06	6.72	7.74	8.61	9.45	9.83	10.56	11.20
11 (8)	0.31	4.18	5.85	6.97	7.49	8.43	10.49	12.69	13.95	16.22	18.35	20.29	21.34	23.10	24.57
12 (9)	0.11	1.33	2.82	4.15	4.86	5.92	8.09	10.17	11.32	13.05	14.77	16.40	17.35	18.89	20.32
13 (10)	0.18	2.61	4.32	5.44	5.90	6.63	8.28	10.04	11.01	12.71	14.33	15.86	16.73	18.22	19.60
MEAN	0.94	5.26	7.31	8.52	9.10	9.79	11.09	12.35	13.16	14.36	15.46	16.51	17.11	18.02	18.83
SD	1.24	4.51	5.22	5.45	5.69	5.75	5.84	5.85	6.00	6.05	6.13	6.24	6.36	6.49	6.63

Concentration-time course of 4-hydroxybenzoic acid (µg equiv/mL)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	0.50	2.31	1.47	0.91	0.65	0.46	0.37	0.27	0.29	0.26	0.22	0.22	0.17	0.19	0.16
3 (2)	1.49	2.83	0.84	0.34	0.17	0.13	0.12	0.10	0.09	0.10	0.09	0.09	0.06	0.07	0.08
4 (3)	1.04	2.03	1.00	0.23	0.12	0.10	0.09	0.10	0.07	0.09	0.08	0.08	0.05	0.06	0.05
5 (4)	0.34	5.46	1.39	0.74	0.38	0.37	0.31	0.26	0.18	0.24	0.21	0.18	0.10	0.12	0.12
6 (5)	0.11	0.41	0.26	0.19	0.12	0.16	0.17	0.19	0.11	0.21	0.19	0.19	0.11	0.15	0.12
8 (6)	0.02	0.51	0.92	0.74	0.15	0.33	0.22	0.29	0.09	0.17	0.16	0.15	0.06	0.12	0.12
10 (7)	0.06	0.91	0.44	0.30	0.07	0.15	0.15	0.11	0.13	0.21	0.18	0.17	0.08	0.15	0.13
11 (8)	0.13	1.57	0.68	0.45	0.21	0.38	0.42	0.44	0.26	0.46	0.43	0.39	0.21	0.36	0.30
12 (9)	0.04	0.49	0.60	0.54	0.29	0.43	0.44	0.42	0.23	0.35	0.35	0.33	0.19	0.31	0.29
13 (10)	0.07	0.99	0.69	0.45	0.19	0.30	0.33	0.36	0.19	0.34	0.33	0.31	0.18	0.30	0.28
MEAN	0.38	1.75	0.83	0.49	0.23	0.28	0.26	0.25	0.16	0.24	0.22	0.21	0.12	0.18	0.16
SD	0.50	1.55	0.39	0.24	0.17	0.14	0.13	0.13	0.08	0.12	0.11	0.10	0.06	0.10	0.09

Cumulative percent of 4-hydroxybenzoic acid absorbed

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	1.93	10.83	16.52	20.01	22.51	24.30	27.18	29.27	31.52	33.56	35.24	36.93	38.28	39.76	40.96
3 (2)	5.77	16.73	19.98	21.29	21.95	22.44	23.39	24.18	24.85	25.61	26.32	27.01	27.52	28.07	28.66
4 (3)	4.09	12.04	15.96	16.86	17.31	17.69	18.41	19.18	19.74	20.44	21.03	21.64	22.01	22.49	22.90
5 (4)	1.32	22.48	27.86	30.72	32.21	33.62	36.01	38.06	39.48	41.31	42.92	44.35	45.14	46.10	47.05
6 (5)	0.44	2.06	3.09	3.83	4.30	4.93	6.28	7.76	8.62	10.28	11.83	13.29	14.15	15.35	16.32
8 (6)	0.09	2.08	5.62	8.47	9.05	10.33	12.03	14.29	14.99	16.33	17.54	18.69	19.19	20.09	20.99
10 (7)	0.23	3.77	5.49	6.66	6.92	7.52	8.67	9.56	10.61	12.22	13.60	14.93	15.52	16.68	17.69
11 (8)	0.49	6.59	9.23	10.99	11.81	13.29	16.54	20.01	22.00	25.58	28.93	31.99	33.65	36.43	38.74
12 (9)	0.17	2.11	4.47	6.58	7.71	9.40	12.84	16.15	17.97	20.71	23.45	26.03	27.54	29.99	32.25
13 (10)	0.28	4.11	6.79	8.55	9.27	10.42	13.02	15.79	17.31	19.98	22.52	24.94	26.31	28.66	30.82
MEAN	1.48	8.28	11.50	13.40	14.30	15.39	17.44	19.43	20.71	22.60	24.34	25.98	26.93	28.36	29.64
SD	1.95	7.07	8.18	8.53	8.90	9.00	9.12	9.13	9.36	9.44	9.56	9.72	9.91	10.12	10.35

APPENDIX C

Methylparaben – Human

Application amounts and rates

Skin Replicate	Activity applied (μCi)	Total MP (μg)	MP per area (μg/cm^2)
1	0.78	39.7	62.0
2	0.74	37.4	58.4
3	0.78	39.7	62.1
4	0.78	39.5	61.8
5	0.78	39.4	61.6
6	0.78	39.4	61.6
7	0.77	39.2	61.2
8	0.80	40.4	63.1
9	0.73	37.0	57.9
10	0.79	40.2	62.9
11	0.80	40.7	63.6
12	0.79	40.3	63.0
13	0.78	39.3	61.5
Mean	0.78	39.41	61.58
SD	0.02	1.09	1.70

Cumulative amount of radioactivity absorbed per area (µg equiv/cm^2)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	3.35	10.54	17.36	22.36	26.39	29.26	33.33	36.52	39.75	45.23	49.12	52.24	55.17	57.07	59.63
2 (2)	2.50	14.94	23.09	27.89	30.48	32.76	35.85	39.06	42.10	45.01	47.74	49.94	52.24	54.21	55.91
3 (3)	3.60	12.50	19.51	23.40	27.33	30.85	36.28	41.86	46.96	50.52	54.58	57.94	60.54	62.13	63.50
4 (4)	0.85	8.30	16.06	20.42	23.57	26.65	31.48	35.51	39.15	41.46	44.09	46.63	48.89	50.99	52.80
5 (5)	1.13	9.69	16.74	21.54	25.06	27.44	30.43	33.32	36.80	40.01	43.44	46.70	49.64	52.75	55.14
1 (6)	5.62	15.23	19.34	21.39	24.02	25.83	29.50	33.22	36.23	38.39	40.67	42.39	44.01	44.95	45.79
2 (7)	3.79	13.70	18.33	20.77	22.60	23.97	25.57	26.81	27.99	29.08	30.35	31.28	32.39	33.19	33.96
3 (8)	2.00	8.88	11.98	14.40	16.53	18.45	21.24	23.56	25.47	27.27	29.04	30.55	31.83	32.87	34.23
4 (9)	2.95	11.98	16.50	18.70	21.18	23.07	26.03	28.34	30.22	31.42	32.86	34.22	35.13	36.36	37.33
5 (10)	1.75	9.57	14.97	18.05	20.03	22.21	25.51	28.34	31.02	33.44	35.61	37.62	39.28	41.00	42.07
1 (11)	2.04	11.14	16.65	20.50	23.24	25.60	29.43	32.85	36.10	39.05	41.47	43.42	45.05	46.46	47.61
2 (12)	0.45	6.12	12.77	18.61	22.90	26.32	31.55	35.34	39.06	42.71	45.56	48.32	50.72	52.70	54.48
3 (13)	1.20	8.54	14.35	18.92	22.41	25.32	30.25	34.25	38.32	41.89	44.93	47.49	49.49	51.20	52.69
MEAN	2.40	10.86	16.74	20.53	23.52	25.98	29.73	33.00	36.09	38.88	41.50	43.75	45.72	47.38	48.86
SD	1.44	2.73	2.96	3.18	3.45	3.73	4.30	5.10	5.99	6.84	7.62	8.29	8.87	9.24	9.61

Concentration-time course of total radioactivity (µg equiv/mL)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	1.36	2.91	2.76	2.02	1.63	1.17	0.82	0.64	0.65	1.11	0.78	0.63	0.59	0.38	0.52
2 (2)	1.01	5.04	3.30	1.95	1.05	0.92	0.62	0.65	0.61	0.59	0.55	0.44	0.46	0.40	0.34
3 (3)	1.46	3.60	2.84	1.58	1.59	1.43	1.10	1.13	1.03	0.72	0.82	0.68	0.52	0.32	0.28
4 (4)	0.34	3.02	3.14	1.76	1.28	1.25	0.97	0.81	0.74	0.47	0.53	0.51	0.46	0.42	0.36
5 (5)	0.46	3.47	2.85	1.95	1.42	0.97	0.60	0.58	0.70	0.65	0.69	0.66	0.59	0.63	0.48
1 (6)	2.28	3.89	1.66	0.83	1.07	0.73	0.74	0.75	0.61	0.44	0.46	0.35	0.33	0.19	0.17
2 (7)	1.54	4.01	1.88	0.99	0.74	0.56	0.32	0.25	0.24	0.22	0.26	0.19	0.23	0.16	0.16
3 (8)	0.81	2.79	1.26	0.98	0.86	0.78	0.56	0.47	0.38	0.36	0.36	0.30	0.26	0.21	0.27
4 (9)	1.20	3.66	1.83	0.89	1.01	0.77	0.60	0.47	0.38	0.24	0.29	0.27	0.18	0.25	0.20
5 (10)	0.71	3.17	2.19	1.25	0.80	0.88	0.67	0.57	0.54	0.49	0.44	0.41	0.34	0.35	0.22
1 (11)	0.83	3.69	2.23	1.56	1.11	0.96	0.77	0.69	0.66	0.59	0.49	0.39	0.33	0.28	0.23
2 (12)	0.18	2.30	2.69	2.36	1.74	1.39	1.06	0.76	0.75	0.74	0.58	0.56	0.48	0.40	0.36
3 (13)	0.49	2.97	2.35	1.85	1.41	1.18	0.99	0.81	0.82	0.72	0.61	0.52	0.40	0.34	0.30
MEAN	0.97	3.42	2.38	1.54	1.21	1.00	0.76	0.66	0.62	0.56	0.53	0.45	0.40	0.33	0.30
SD	0.58	0.69	0.61	0.50	0.33	0.27	0.23	0.21	0.21	0.24	0.17	0.15	0.13	0.12	0.11

Cumulative percent of radioactivity absorbed

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	5.40	17.00	27.99	36.05	42.55	47.19	53.75	58.89	64.11	72.94	79.20	84.24	88.97	92.03	96.16
2 (2)	4.29	25.58	39.52	47.75	52.19	56.09	61.38	66.88	72.07	77.06	81.74	85.49	89.44	92.82	95.73
3 (3)	5.80	20.13	31.42	37.69	44.02	49.69	58.44	67.42	75.63	81.38	87.91	93.33	97.51	100.07	102.28
4 (4)	1.38	13.44	26.00	33.06	38.17	43.16	50.97	57.49	63.40	67.13	71.39	75.51	79.17	82.57	85.49
5 (5)	1.84	15.74	27.18	34.99	40.69	44.57	49.43	54.12	59.76	64.98	70.55	75.84	80.62	85.68	89.54
1 (6)	9.12	24.72	31.39	34.71	38.98	41.92	47.88	53.91	58.79	62.30	66.01	68.79	71.42	72.94	74.30
2 (7)	6.20	22.38	29.96	33.94	36.92	39.17	41.79	43.82	45.75	47.52	49.60	51.11	52.94	54.23	55.50
3 (8)	3.16	14.07	18.99	22.82	26.20	29.25	33.66	37.34	40.36	43.22	46.03	48.41	50.44	52.10	54.25
4 (9)	5.11	20.71	28.52	32.31	36.60	39.86	44.98	48.98	52.23	54.30	56.79	59.14	60.71	62.84	64.51
5 (10)	2.78	15.23	23.82	28.72	31.87	35.34	40.59	45.08	49.35	53.20	56.65	59.85	62.49	65.23	66.93
1 (11)	3.21	17.51	26.17	32.22	36.52	40.23	46.25	51.63	56.74	61.36	65.17	68.23	70.80	73.01	74.82
2 (12)	0.72	9.72	20.27	29.53	36.33	41.77	50.07	56.07	61.99	67.77	72.29	76.68	80.49	83.63	86.45
3 (13)	1.95	13.89	23.34	30.78	36.45	41.19	49.20	55.71	62.33	68.14	73.09	77.25	80.51	83.29	85.71
MEAN	3.92	17.70	27.27	33.43	38.27	42.26	48.34	53.64	58.65	63.18	67.42	71.07	74.27	76.96	79.36
SD	2.36	4.73	5.33	5.73	6.14	6.55	7.35	8.56	9.91	11.22	12.44	13.49	14.42	15.02	15.62

Material Balance

Cell ID (replicate)	Receptor Fluid	Receptor wash	Skin Wash	Donor Chamber	Tape Strips	Skin	Total Recovery
1 (1)	96.16	0.41	8.89	0.02	0.70	4.09	110.27
2 (2)	95.73	0.30	16.39	0.84	0.99	3.41	117.66
3 (3)	102.28	0.46	10.16	0.09	0.28	4.22	117.49
4 (4)	85.49	0.68	9.93	0.03	0.74	6.12	103.00
5 (5)	89.54	0.47	12.61	0.03	0.75	4.39	107.79
1 (6)	74.30	0.45	15.33	0.03	0.20	3.73	94.04
2 (7)	55.50	0.33	23.12	3.46	34.60	4.27	121.27
3 (8)	54.25	0.69	40.77	0.22	31.25	8.47	135.65
4 (9)	64.51	0.43	10.87	0.43	4.69	4.08	85.03
5 (10)	66.93	0.42	9.29	0.04	4.67	4.01	85.36
1 (11)	74.82	0.46	11.39	0.01	0.17	2.21	89.06
2 (12)	86.45	0.47	11.80	0.25	0.36	9.38	108.72
3 (13)	85.71	0.39	9.92	0.07	0.32	5.00	101.42
MEAN	79.36	0.46	14.65	0.42	6.13	4.88	105.91
SD	15.62	0.11	8.76	0.94	12.01	2.01	15.10

Cell ID (replicate)	Absorbed	Absorbable	Unabsorbed
1 (1)	96.57	100.66	9.61
2 (2)	96.03	99.44	18.22
3 (3)	102.74	106.96	10.53
4 (4)	86.17	92.29	10.71
5 (5)	90.02	94.41	13.38
1 (6)	74.75	78.48	15.56
2 (7)	55.83	60.10	61.18
3 (8)	54.94	63.41	72.25
4 (9)	64.95	69.03	16.00
5 (10)	67.35	71.36	14.00
1 (11)	75.28	77.49	11.57
2 (12)	86.92	96.30	12.41
3 (13)	86.10	91.10	10.32
MEAN	79.82	84.69	21.21
SD	15.60	15.46	20.48

Cumulative amount of methylparaben absorbed per area (μg/cm^2)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	3.50	4.30	9.35	11.41	11.79	12.71	14.29	15.72	16.85	18.91	20.71	22.50	23.13	24.27	25.23
2 (2)	0.85	1.62	1.81	3.03	3.34	4.05	5.24	6.50	7.47	8.73	9.82	10.88	11.68	12.33	12.89
3 (3)	1.09	1.70	3.84	4.92	5.25	6.25	8.01	9.82	11.29	12.77	14.17	15.20	15.82	16.35	16.67
4 (4)	0.42	0.85	3.88	5.19	5.66	6.37	7.40	8.57	9.52	10.47	11.45	12.28	12.77	13.26	13.67
5 (5)	0.51	0.92	3.91	4.93	5.35	5.85	6.64	7.46	8.16	9.24	10.31	11.23	12.06	12.79	13.34
1 (6)	6.97	26.88	30.59	33.20	35.30	37.00	39.53	42.06	45.22	47.48	49.25	50.82	52.18	53.00	53.69
2 (7)	4.22	22.23	26.28	28.03	29.07	29.74	30.87	31.70	32.71	33.57	34.40	35.19	35.79	36.41	37.01
3 (8)	3.18	15.61	19.52	21.78	23.50	24.72	26.45	27.97	29.11	30.16	31.18	32.01	32.79	33.33	33.95
4 (9)	2.28	13.31	16.16	17.63	19.01	19.94	21.42	22.75	24.02	25.10	26.06	26.92	27.63	28.39	28.89
5 (10)	1.82	17.42	21.87	24.02	25.76	27.00	29.04	30.85	32.69	34.19	35.49	36.59	37.72	38.59	39.36
1 (11)	2.41	7.64	14.13	17.83	20.37	22.25	25.31	29.00	31.75	34.29	36.22	37.69	39.00	39.88	40.61
2 (12)	0.57	3.14	9.93	14.57	17.40	19.27	21.94	25.64	28.05	30.15	31.86	33.39	34.34	35.16	35.78
3 (13)	1.03	8.20	13.06	15.96	17.90	19.40	21.78	24.73	26.91	28.73	30.20	31.33	32.17	32.66	33.16
MEAN	2.22	9.52	13.41	15.58	16.90	18.04	19.84	21.75	23.36	24.91	26.24	27.39	28.24	28.96	29.56
SD	1.89	8.77	9.19	9.55	10.13	10.38	10.77	11.18	11.74	12.00	12.15	12.28	12.47	12.52	12.60

Concentration-time course of methylparaben (µg equiv/mL)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	1.42	0.32	2.04	0.84	0.15	0.37	0.32	0.29	0.23	0.42	0.36	0.36	0.13	0.23	0.19
2 (2)	0.34	0.31	0.08	0.49	0.13	0.29	0.24	0.25	0.20	0.25	0.22	0.21	0.16	0.13	0.11
3 (3)	0.44	0.25	0.87	0.44	0.14	0.40	0.36	0.37	0.30	0.30	0.28	0.21	0.12	0.11	0.06
4 (4)	0.17	0.17	1.23	0.53	0.19	0.29	0.21	0.24	0.19	0.19	0.20	0.17	0.10	0.10	0.08
5 (5)	0.21	0.16	1.21	0.41	0.17	0.21	0.16	0.16	0.14	0.22	0.21	0.19	0.17	0.15	0.11
1 (6)	2.82	8.06	1.50	1.06	0.85	0.69	0.51	0.51	0.64	0.46	0.36	0.32	0.27	0.17	0.14
2 (7)	1.71	7.29	1.64	0.71	0.42	0.27	0.23	0.17	0.20	0.17	0.17	0.16	0.12	0.12	0.12
3 (8)	1.29	5.04	1.59	0.91	0.70	0.50	0.35	0.31	0.23	0.21	0.21	0.17	0.16	0.11	0.12
4 (9)	0.92	4.47	1.15	0.60	0.56	0.38	0.30	0.27	0.26	0.22	0.19	0.17	0.14	0.15	0.10
5 (10)	0.74	6.32	1.80	0.87	0.71	0.50	0.41	0.37	0.37	0.30	0.26	0.22	0.23	0.18	0.16
1 (11)	0.98	2.12	2.63	1.50	1.03	0.76	0.62	0.75	0.56	0.51	0.39	0.30	0.26	0.18	0.15
2 (12)	0.23	1.04	2.75	1.88	1.15	0.75	0.54	0.75	0.49	0.43	0.34	0.31	0.19	0.17	0.13
3 (13)	0.42	2.90	1.97	1.17	0.79	0.61	0.48	0.60	0.44	0.37	0.30	0.23	0.17	0.10	0.10
MEAN	0.90	2.96	1.57	0.88	0.54	0.46	0.36	0.39	0.33	0.31	0.27	0.23	0.17	0.15	0.12
SD	0.77	2.94	0.71	0.44	0.36	0.19	0.14	0.20	0.16	0.11	0.08	0.07	0.05	0.04	0.03

Cumulative percent of methylparaben absorbed

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	5.65	6.94	15.08	18.41	19.02	20.50	23.04	25.35	27.17	30.49	33.40	36.29	37.30	39.14	40.69
2 (2)	1.45	2.78	3.10	5.19	5.72	6.94	8.98	11.13	12.79	14.95	16.82	18.63	19.99	21.12	22.06
3 (3)	1.75	2.73	6.18	7.92	8.46	10.06	12.90	15.82	18.18	20.57	22.83	24.48	25.47	26.33	26.85
4 (4)	0.68	1.37	6.28	8.41	9.17	10.32	11.98	13.88	15.42	16.95	18.55	19.88	20.67	21.48	22.13
5 (5)	0.83	1.49	6.34	8.00	8.68	9.51	10.79	12.11	13.25	15.01	16.74	18.24	19.58	20.78	21.67
1 (6)	11.31	43.62	49.64	53.88	57.28	60.05	64.16	68.25	73.37	77.05	79.92	82.47	84.67	86.01	87.13
2 (7)	6.90	36.32	42.95	45.80	47.51	48.60	50.44	51.80	53.44	54.85	56.22	57.50	58.49	59.49	60.48
3 (8)	5.03	24.74	30.94	34.52	37.25	39.19	41.92	44.32	46.14	47.80	49.42	50.73	51.97	52.83	53.80
4 (9)	3.95	23.01	27.92	30.46	32.85	34.46	37.01	39.31	41.52	43.38	45.04	46.53	47.75	49.06	49.92
5 (10)	2.90	27.71	34.80	38.21	40.98	42.96	46.20	49.08	52.01	54.39	56.46	58.22	60.01	61.39	62.62
1 (11)	3.79	12.00	22.20	28.01	32.00	34.97	39.77	45.58	49.90	53.89	56.93	59.24	61.29	62.67	63.81
2 (12)	0.90	4.98	15.77	23.12	27.62	30.58	34.82	40.68	44.50	47.85	50.55	52.99	54.49	55.79	56.78
3 (13)	1.68	13.34	21.25	25.95	29.11	31.56	35.43	40.22	43.77	46.73	49.12	50.97	52.33	53.13	53.94
MEAN	3.60	15.46	21.73	25.22	27.36	29.21	32.11	35.20	37.81	40.30	42.46	44.32	45.69	46.86	47.84
SD	3.06	14.25	14.91	15.46	16.36	16.75	17.33	17.94	18.82	19.20	19.41	19.59	19.88	19.96	20.07

Cumulative amount of 4-hydroxybenzoic acid absorbed per area (µg/cm^2)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	0.80	5.22	8.36	10.66	12.65	13.84	15.79	17.61	18.77	21.82	23.57	25.30	26.63	27.59	28.43
2 (2)	1.00	7.82	12.52	15.12	16.44	17.57	19.19	20.73	21.77	23.25	24.47	25.72	26.31	27.09	27.70
3 (3)	0.55	7.62	11.76	14.23	15.88	17.37	19.96	22.69	24.23	26.53	28.60	30.35	31.09	32.00	32.57
4 (4)	0.32	4.76	9.53	12.78	14.44	15.80	17.80	19.78	20.95	22.54	24.21	25.68	26.40	27.40	28.24
5 (5)	0.21	3.55	7.11	9.51	10.57	11.87	13.55	15.27	16.62	18.99	20.93	22.61	24.08	25.45	26.38
1 (6)	0.32	0.94	1.75	2.39	2.93	3.32	3.98	4.77	5.72	6.39	7.03	7.71	8.29	8.63	8.94
2 (7)	0.30	1.07	2.01	2.69	3.23	3.56	4.03	4.37	4.72	4.99	5.22	5.46	5.73	5.91	6.04
3 (8)	0.15	0.66	1.37	1.96	2.43	2.76	3.24	3.64	4.05	4.42	4.82	5.19	5.62	5.92	6.22
4 (9)	0.16	0.82	1.63	2.20	2.80	3.18	3.81	4.26	4.68	5.05	5.38	5.72	6.03	6.37	6.64
5 (10)	0.10	0.61	1.47	2.10	2.66	3.03	3.63	4.22	4.89	5.59	6.14	6.66	7.11	7.37	7.59
1 (11)	0.11	1.30	2.13	2.95	3.60	4.09	4.92	5.85	6.88	7.98	8.89	9.61	10.29	10.82	11.23
2 (12)	0.05	1.22	2.40	3.78	5.00	6.06	7.75	9.76	11.53	13.25	14.69	16.11	17.02	17.89	18.70
3 (13)	0.09	0.65	1.53	2.53	3.42	4.11	5.19	6.54	7.87	9.20	10.43	11.66	12.51	13.14	13.73
MEAN	0.32	2.79	4.89	6.38	7.39	8.20	9.45	10.73	11.75	13.08	14.18	15.21	15.93	16.58	17.11
SD	0.29	2.70	4.30	5.21	5.65	6.06	6.69	7.34	7.61	8.33	8.91	9.42	9.65	9.97	10.19

Concentration-time course of 4-hydroxybenzoic acid (µg equiv/mL)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	0.32	1.79	1.27	0.93	0.81	0.48	0.40	0.37	0.24	0.61	0.35	0.35	0.27	0.19	0.17
2 (2)	0.40	2.76	1.90	1.05	0.54	0.46	0.33	0.31	0.21	0.30	0.25	0.25	0.12	0.16	0.12
3 (3)	0.22	2.86	1.67	1.00	0.67	0.60	0.52	0.55	0.31	0.46	0.42	0.35	0.15	0.18	0.11
4 (4)	0.13	1.80	1.93	1.32	0.67	0.55	0.40	0.40	0.24	0.32	0.34	0.30	0.14	0.20	0.17
5 (5)	0.08	1.35	1.44	0.97	0.43	0.53	0.34	0.35	0.27	0.48	0.39	0.34	0.30	0.28	0.19
1 (6)	0.13	0.25	0.33	0.26	0.22	0.16	0.13	0.16	0.19	0.13	0.13	0.14	0.12	0.07	0.06
2 (7)	0.12	0.31	0.38	0.27	0.22	0.13	0.09	0.07	0.07	0.05	0.05	0.05	0.05	0.04	0.03
3 (8)	0.06	0.21	0.29	0.24	0.19	0.13	0.10	0.08	0.08	0.07	0.08	0.08	0.09	0.06	0.06
4 (9)	0.06	0.27	0.33	0.23	0.24	0.16	0.13	0.09	0.09	0.07	0.07	0.07	0.06	0.07	0.05
5 (10)	0.04	0.21	0.35	0.26	0.22	0.15	0.12	0.12	0.13	0.14	0.11	0.11	0.09	0.05	0.04
1 (11)	0.04	0.48	0.33	0.33	0.26	0.20	0.17	0.19	0.21	0.22	0.18	0.15	0.14	0.11	0.08
2 (12)	0.02	0.48	0.48	0.56	0.49	0.43	0.34	0.41	0.36	0.35	0.29	0.29	0.18	0.17	0.16
3 (13)	0.04	0.23	0.36	0.41	0.36	0.28	0.22	0.27	0.27	0.27	0.25	0.25	0.17	0.13	0.12
MEAN	0.13	1.00	0.85	0.60	0.41	0.33	0.25	0.26	0.21	0.27	0.22	0.21	0.15	0.13	0.11
SD	0.12	1.00	0.67	0.39	0.21	0.18	0.14	0.15	0.09	0.18	0.13	0.11	0.07	0.07	0.06

Cumulative percent of 4-hydroxybenzoic acid absorbed

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1 (1)	1.29	8.42	13.48	17.19	20.40	22.31	25.47	28.39	30.27	35.18	38.01	40.80	42.94	44.48	45.84
2 (2)	1.71	13.38	21.43	25.88	28.15	30.09	32.85	35.49	37.28	39.81	41.89	44.04	45.05	46.39	47.43
3 (3)	0.89	12.28	18.93	22.93	25.58	27.98	32.15	36.55	39.03	42.73	46.07	48.88	50.07	51.55	52.46
4 (4)	0.52	7.71	15.43	20.70	23.38	25.58	28.83	32.03	33.92	36.50	39.20	41.59	42.75	44.36	45.73
5 (5)	0.34	5.76	11.55	15.44	17.17	19.28	22.01	24.80	26.99	30.85	34.00	36.71	39.10	41.32	42.85
1 (6)	0.52	1.52	2.85	3.88	4.76	5.39	6.47	7.74	9.28	10.36	11.40	12.51	13.46	14.01	14.51
2 (7)	0.48	1.75	3.29	4.39	5.28	5.82	6.58	7.14	7.72	8.15	8.54	8.92	9.36	9.66	9.86
3 (8)	0.24	1.05	2.17	3.10	3.85	4.37	5.13	5.76	6.42	7.00	7.64	8.22	8.90	9.38	9.85
4 (9)	0.27	1.42	2.81	3.80	4.84	5.50	6.59	7.35	8.09	8.72	9.30	9.88	10.42	11.02	11.48
5 (10)	0.16	0.97	2.34	3.35	4.23	4.81	5.77	6.72	7.77	8.90	9.77	10.59	11.31	11.72	12.07
1 (11)	0.17	2.04	3.34	4.63	5.66	6.42	7.73	9.19	10.81	12.55	13.97	15.10	16.17	17.00	17.65
2 (12)	0.08	1.94	3.81	6.00	7.94	9.61	12.29	15.48	18.30	21.03	23.32	25.56	27.01	28.38	29.67
3 (13)	0.14	1.05	2.49	4.12	5.56	6.68	8.44	10.65	12.81	14.97	16.97	18.97	20.35	21.37	22.33
MEAN	0.52	4.56	7.99	10.42	12.06	13.37	15.41	17.48	19.13	21.29	23.08	24.75	25.92	26.97	27.83
SD	0.49	4.48	7.12	8.62	9.34	10.00	11.03	12.07	12.52	13.67	14.60	15.44	15.80	16.31	16.68

APPENDIX D

Butylparaben – Rat

Application amounts and rates

Skin Replicate	Activity applied (μCi)	Total BP (μg)	BP per area (μg/cm^2)
1	0.87	20.9	32.6
2	0.95	22.9	35.8
3	0.95	22.9	35.7
4	0.98	23.6	36.8
5	0.97	23.3	36.4
6	0.98	23.6	36.8
7	0.98	23.5	36.7
8	0.96	23.1	36.0
9	0.98	23.6	36.9
10	0.97	23.3	36.4
Mean	0.96	23.06	36.03
SD	0.03	0.82	1.27

Cumulative amount of radioactivity absorbed per area (µg equiv/cm^2)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
2 (1)	0.21	1.79	3.19	4.75	6.31	7.20	9.31	11.01	12.15	13.46	14.75	15.70	16.55	17.28	17.91
3 (2)	0.35	1.94	3.53	4.90	6.40	7.87	10.28	12.62	14.67	16.64	18.33	19.80	21.11	22.23	23.12
4 (3)	0.81	3.35	5.39	7.12	8.18	9.84	12.70	15.38	17.13	18.82	20.56	22.09	23.18	24.34	25.23
5 (4)	0.35	3.36	7.22	10.18	11.82	13.32	14.86	16.57	18.05	19.16	19.92	20.74	21.39	21.93	22.37
6 (5)	0.56	2.85	3.97	4.83	5.68	6.41	7.55	8.98	10.32	11.55	12.67	13.68	14.56	15.45	16.25
8 (6)	0.33	2.37	4.87	6.67	8.38	9.69	11.96	14.03	15.73	17.15	18.45	19.48	20.28	21.23	21.87
9 (7)	0.41	3.45	6.20	8.38	9.77	10.83	12.32	13.52	14.53	15.47	16.29	16.99	17.62	18.16	18.64
11 (8)	0.11	0.96	2.12	2.99	3.85	4.64	5.94	7.19	8.37	9.44	10.48	11.35	12.23	12.88	13.54
12 (9)	0.17	2.52	5.15	7.46	8.46	9.59	11.54	13.02	14.08	15.22	16.27	17.16	17.94	18.65	19.25
13 (10)	0.04	0.99	2.42	3.56	4.62	5.63	7.28	8.89	10.39	11.80	13.12	14.22	15.29	16.18	17.10
MEAN	0.33	2.36	4.41	6.08	7.35	8.50	10.37	12.12	13.54	14.87	16.08	17.12	18.02	18.83	19.53
SD	0.23	0.93	1.65	2.26	2.43	2.64	2.82	3.03	3.15	3.24	3.32	3.43	3.46	3.56	3.57

Concentration-time course of total radioactivity (µg equiv/mL)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
2 (1)	0.08	0.64	0.57	0.63	0.63	0.36	0.43	0.34	0.23	0.26	0.26	0.19	0.17	0.15	0.13
3 (2)	0.14	0.64	0.65	0.55	0.61	0.60	0.48	0.47	0.41	0.40	0.34	0.30	0.27	0.23	0.18
4 (3)	0.33	1.03	0.83	0.70	0.43	0.67	0.58	0.54	0.36	0.34	0.35	0.31	0.22	0.23	0.18
5 (4)	0.14	1.22	1.57	1.20	0.66	0.61	0.31	0.34	0.30	0.22	0.15	0.16	0.13	0.11	0.09
6 (5)	0.23	0.93	0.45	0.35	0.34	0.30	0.23	0.29	0.27	0.25	0.23	0.20	0.18	0.18	0.16
8 (6)	0.13	0.83	1.01	0.73	0.69	0.53	0.46	0.42	0.34	0.29	0.26	0.21	0.16	0.19	0.13
9 (7)	0.17	1.23	1.11	0.88	0.56	0.43	0.30	0.24	0.20	0.19	0.17	0.14	0.13	0.11	0.10
11 (8)	0.04	0.34	0.47	0.35	0.35	0.32	0.26	0.25	0.24	0.22	0.21	0.18	0.18	0.13	0.13
12 (9)	0.07	0.95	1.06	0.94	0.41	0.46	0.39	0.30	0.21	0.23	0.21	0.18	0.16	0.14	0.12
13 (10)	0.02	0.39	0.58	0.46	0.43	0.41	0.33	0.32	0.30	0.28	0.27	0.22	0.22	0.18	0.19
MEAN	0.14	0.82	0.83	0.68	0.51	0.47	0.38	0.35	0.29	0.27	0.24	0.21	0.18	0.16	0.14
SD	0.09	0.31	0.36	0.27	0.13	0.13	0.11	0.10	0.07	0.06	0.07	0.05	0.04	0.04	0.03

Cumulative percent of radioactivity absorbed

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
2 (1)	0.63	5.49	9.78	14.56	19.34	22.07	28.54	33.77	37.24	41.26	45.24	48.14	50.76	52.98	54.91
3 (2)	0.98	5.41	9.86	13.66	17.86	21.98	28.67	35.21	40.94	46.43	51.16	55.24	58.91	62.04	64.52
4 (3)	2.27	9.38	15.10	19.94	22.91	27.54	35.54	43.04	47.97	52.69	57.54	61.84	64.88	68.14	70.62
5 (4)	0.95	9.12	19.61	27.64	32.10	36.18	40.36	44.99	49.01	52.02	54.10	56.31	58.09	59.54	60.75
6 (5)	1.54	7.84	10.92	13.27	15.61	17.63	20.75	24.69	28.37	31.75	34.83	37.60	40.02	42.45	44.67
8 (6)	0.89	6.42	13.22	18.11	22.76	26.31	32.47	38.10	42.70	46.58	50.09	52.90	55.07	57.66	59.38
9 (7)	1.12	9.41	16.89	22.83	26.62	29.53	33.58	36.85	39.61	42.16	44.39	46.30	48.03	49.49	50.80
11 (8)	0.30	2.66	5.89	8.31	10.67	12.87	16.48	19.97	23.24	26.21	29.09	31.51	33.96	35.75	37.58
12 (9)	0.47	6.82	13.93	20.18	22.90	25.95	31.23	35.23	38.11	41.21	44.03	46.44	48.55	50.47	52.11
13 (10)	0.11	2.73	6.65	9.79	12.71	15.48	20.02	24.43	28.56	32.42	36.04	39.09	42.03	44.48	47.00
MEAN	0.93	6.53	12.18	16.83	20.35	23.55	28.76	33.63	37.58	41.27	44.65	47.54	50.03	52.30	54.23
SD	0.63	2.51	4.38	6.01	6.48	7.02	7.57	8.19	8.51	8.80	9.07	9.43	9.54	9.84	9.89

Material Balance

Cell ID (replicate)	Receptor Fluid	Receptor wash	Skin Wash	Donor Chamber	Tape Strips	Skin	Total Recovery
2 (1)	54.91	0.47	27.35	3.02	8.76	11.89	106.40
3 (2)	64.52	0.50	14.04	0.03	14.14	8.27	101.49
4 (3)	70.62	0.49	12.20	0.05	9.23	10.73	103.32
5 (4)	60.75	0.28	10.82	0.01	12.25	2.36	86.47
6 (5)	44.67	0.44	25.22	0.89	16.51	7.61	95.33
8 (6)	59.38	0.49	13.75	0.17	13.10	14.69	101.58
9 (7)	50.80	0.33	16.54	0.03	20.70	6.84	95.24
11 (8)	37.58	0.55	25.42	0.06	0.91	36.57	101.10
12 (9)	52.11	0.29	14.06	0.07	10.34	18.85	95.73
13 (10)	47.00	0.55	23.49	0.02	16.74	12.31	100.12
MEAN	54.23	0.44	18.29	0.44	12.27	13.01	98.68
SD	9.89	0.10	6.33	0.95	5.45	9.44	5.64

Cell ID (replicate)	Absorbed	Absorbable	Unabsorbed
2 (1)	55.38	67.28	39.13
3 (2)	65.02	73.29	28.21
4 (3)	71.11	81.84	21.48
5 (4)	61.03	63.39	23.08
6 (5)	45.11	52.72	42.62
8 (6)	59.87	74.56	27.02
9 (7)	51.13	57.96	37.28
11 (8)	38.13	74.70	26.39
12 (9)	52.41	71.25	24.47
13 (10)	47.55	59.87	40.25
MEAN	54.67	67.69	30.99
SD	9.88	9.06	7.94

Cumulative amount of butylparaben absorbed per area (µg/cm^2)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
2 (1)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.04	0.05	0.07	0.07	0.08	0.10	0.10
3 (2)	0.06	0.13	0.23	0.30	0.40	0.47	0.60	0.72	0.86	0.96	1.11	1.28	1.46	1.55	1.59
4 (3)	0.11	0.19	0.34	0.47	0.59	0.67	0.82	0.96	1.13	1.27	1.36	1.49	1.63	1.67	1.69
5 (4)	0.04	0.15	0.38	0.52	0.59	0.64	0.74	0.79	0.88	0.91	0.95	0.98	1.01	1.03	1.04
6 (5)	0.20	0.40	0.58	0.69	0.75	0.81	0.91	1.03	1.30	1.48	1.60	1.74	1.85	1.96	2.05
8 (6)	0.02	0.11	0.17	0.21	0.23	0.26	0.31	0.36	0.39	0.43	0.46	0.49	0.52	0.55	0.57
9 (7)	0.07	0.42	0.77	0.96	1.05	1.11	1.22	1.33	1.44	1.54	1.62	1.70	1.76	1.81	1.86
11 (8)	0.01	0.06	0.09	0.12	0.14	0.17	0.21	0.26	0.31	0.36	0.41	0.45	0.49	0.53	0.57
12 (9)	0.01	0.13	0.24	0.31	0.34	0.37	0.42	0.46	0.48	0.52	0.55	0.59	0.62	0.65	0.67
13 (10)	0.01	0.07	0.15	0.19	0.23	0.26	0.32	0.37	0.44	0.50	0.55	0.60	0.65	0.68	0.71
MEAN	0.05	0.17	0.30	0.38	0.44	0.48	0.56	0.63	0.73	0.80	0.87	0.94	1.01	1.05	1.08
SD	0.06	0.14	0.23	0.28	0.31	0.33	0.37	0.40	0.46	0.51	0.54	0.58	0.63	0.65	0.66

Concentration-time course of butylparaben (µg equiv/mL)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
2 (1)	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3 (2)	0.03	0.03	0.04	0.03	0.04	0.03	0.03	0.02	0.03	0.02	0.03	0.03	0.04	0.02	0.01
4 (3)	0.04	0.03	0.06	0.05	0.05	0.03	0.03	0.03	0.04	0.03	0.02	0.03	0.03	0.01	0.00
5 (4)	0.02	0.04	0.09	0.06	0.03	0.02	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.00	0.00
6 (5)	0.08	0.08	0.08	0.04	0.03	0.02	0.02	0.02	0.05	0.04	0.02	0.03	0.02	0.02	0.02
8 (6)	0.01	0.03	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00
9 (7)	0.03	0.14	0.14	0.08	0.04	0.03	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01
11 (8)	0.00	0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
12 (9)	0.01	0.05	0.04	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00
13 (10)	0.00	0.03	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
MEAN	0.02	0.05	0.05	0.03	0.02	0.02	0.02	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01
SD	0.02	0.04	0.04	0.02	0.02	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01

Cumulative percent of butylparaben absorbed

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
2 (1)	0.05	0.05	0.05	0.05	0.06	0.06	0.06	0.10	0.12	0.16	0.22	0.23	0.24	0.30	0.31
3 (2)	0.18	0.37	0.63	0.83	1.12	1.30	1.67	2.00	2.40	2.67	3.10	3.58	4.08	4.33	4.45
4 (3)	0.31	0.53	0.97	1.31	1.66	1.89	2.30	2.69	3.18	3.54	3.81	4.18	4.57	4.68	4.72
5 (4)	0.12	0.40	1.03	1.42	1.59	1.74	2.01	2.15	2.38	2.48	2.58	2.66	2.73	2.79	2.83
6 (5)	0.54	1.09	1.60	1.89	2.07	2.22	2.50	2.83	3.57	4.06	4.40	4.78	5.09	5.39	5.64
8 (6)	0.06	0.29	0.46	0.57	0.64	0.70	0.83	0.97	1.07	1.16	1.26	1.34	1.41	1.49	1.55
9 (7)	0.19	1.16	2.10	2.61	2.86	3.03	3.34	3.63	3.92	4.19	4.43	4.63	4.80	4.94	5.07
11 (8)	0.02	0.16	0.26	0.35	0.40	0.46	0.58	0.72	0.85	0.99	1.12	1.25	1.36	1.47	1.57
12 (9)	0.03	0.35	0.65	0.83	0.93	1.00	1.13	1.23	1.31	1.41	1.50	1.60	1.68	1.75	1.81
13 (10)	0.02	0.21	0.40	0.53	0.62	0.71	0.87	1.01	1.20	1.38	1.52	1.65	1.77	1.86	1.95
MEAN	0.15	0.46	0.82	1.04	1.19	1.31	1.53	1.73	2.00	2.20	2.39	2.59	2.77	2.90	2.99
SD	0.17	0.38	0.63	0.78	0.85	0.91	1.01	1.11	1.28	1.39	1.48	1.61	1.73	1.79	1.83

Cumulative amount of 4-hydroxybenzoic acid absorbed per area (µg/cm^2)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
2 (1)	0.17	0.80	2.08	3.21	4.36	4.93	6.29	7.20	8.32	9.36	10.46	11.29	11.91	12.39	12.83
3 (2)	0.18	1.62	2.61	3.38	4.20	4.87	6.03	7.07	8.26	9.45	10.16	10.64	11.15	11.29	11.76
4 (3)	0.20	1.82	2.98	3.79	4.76	5.50	6.74	8.01	9.35	10.56	11.41	12.06	12.43	12.66	12.91
5 (4)	0.17	2.16	3.88	5.18	6.07	6.71	7.45	7.92	8.51	8.95	9.39	9.64	9.83	10.03	10.26
6 (5)	0.20	1.38	1.78	2.06	2.47	2.71	3.22	3.68	4.27	4.80	5.04	5.45	5.85	6.14	6.28
8 (6)	0.18	1.22	2.28	3.13	3.95	4.64	5.69	6.63	7.40	8.18	8.86	9.46	10.01	10.50	10.99
9 (7)	0.11	1.00	2.28	3.28	4.22	4.74	5.31	5.82	6.38	6.93	7.34	7.72	7.94	8.18	8.47
11 (8)	0.07	0.60	1.18	1.65	2.11	2.49	3.07	3.71	4.40	5.03	5.70	6.38	6.95	7.35	7.76
12 (9)	0.09	1.23	2.78	4.12	5.27	6.00	7.08	7.86	8.78	9.48	10.13	10.69	11.18	11.51	11.86
13 (10)	0.01	0.41	1.06	1.63	2.23	2.74	3.46	4.22	4.98	5.76	6.57	7.27	7.86	8.29	8.65
MEAN	0.14	1.22	2.29	3.14	3.96	4.53	5.43	6.21	7.07	7.85	8.51	9.06	9.51	9.83	10.18
SD	0.06	0.55	0.84	1.12	1.32	1.45	1.63	1.75	1.92	2.07	2.20	2.23	2.24	2.23	2.28

Concentration-time course of 4-hydroxybenzoic acid (µg equiv/mL)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
2 (1)	0.07	0.26	0.52	0.45	0.47	0.23	0.27	0.18	0.23	0.21	0.22	0.17	0.13	0.10	0.09
3 (2)	0.07	0.59	0.40	0.31	0.33	0.27	0.23	0.21	0.24	0.24	0.14	0.10	0.10	0.03	0.09
4 (3)	0.08	0.65	0.47	0.33	0.39	0.30	0.25	0.26	0.27	0.24	0.17	0.13	0.07	0.05	0.05
5 (4)	0.07	0.81	0.70	0.53	0.36	0.26	0.15	0.10	0.12	0.09	0.09	0.05	0.04	0.04	0.05
6 (5)	0.08	0.48	0.16	0.11	0.17	0.10	0.10	0.09	0.12	0.11	0.05	0.08	0.08	0.06	0.03
8 (6)	0.07	0.42	0.43	0.35	0.33	0.28	0.21	0.19	0.16	0.16	0.14	0.12	0.11	0.10	0.10
9 (7)	0.04	0.36	0.52	0.41	0.38	0.21	0.11	0.10	0.11	0.11	0.08	0.08	0.04	0.05	0.06
11 (8)	0.03	0.21	0.23	0.19	0.19	0.15	0.12	0.13	0.14	0.13	0.14	0.14	0.12	0.08	0.08
12 (9)	0.03	0.46	0.63	0.54	0.47	0.30	0.22	0.16	0.19	0.14	0.13	0.11	0.10	0.07	0.07
13 (10)	0.01	0.16	0.26	0.23	0.24	0.21	0.15	0.15	0.15	0.16	0.16	0.14	0.12	0.09	0.07
MEAN	0.06	0.44	0.43	0.35	0.33	0.23	0.18	0.16	0.17	0.16	0.13	0.11	0.09	0.07	0.07
SD	0.03	0.20	0.17	0.14	0.11	0.07	0.06	0.05	0.06	0.06	0.05	0.04	0.03	0.03	0.02

Cumulative percent of 4-hydroxybenzoic acid absorbed

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
2 (1)	0.51	2.44	6.39	9.83	13.36	15.11	19.28	22.09	25.52	28.70	32.08	34.62	36.52	38.00	39.35
3 (2)	0.49	4.52	7.28	9.43	11.72	13.59	16.83	19.73	23.06	26.37	28.36	29.70	31.13	31.51	32.80
4 (3)	0.57	5.09	8.35	10.60	13.33	15.41	18.86	22.42	26.18	29.57	31.93	33.77	34.80	35.43	36.13
5 (4)	0.46	5.87	10.53	14.05	16.50	18.23	20.23	21.51	23.11	24.29	25.51	26.19	26.70	27.24	27.87
6 (5)	0.56	3.81	4.90	5.67	6.80	7.44	8.85	10.13	11.72	13.20	13.86	14.98	16.08	16.87	17.26
8 (6)	0.48	3.31	6.19	8.51	10.71	12.61	15.44	18.00	20.10	22.21	24.05	25.68	27.17	28.52	29.84
9 (7)	0.30	2.71	6.20	8.94	11.51	12.93	14.47	15.86	17.39	18.88	20.02	21.05	21.65	22.31	23.07
11 (8)	0.19	1.66	3.26	4.59	5.86	6.91	8.53	10.28	12.22	13.96	15.82	17.70	19.29	20.41	21.54
12 (9)	0.23	3.33	7.53	11.15	14.26	16.24	19.16	21.28	23.76	25.65	27.41	28.93	30.26	31.15	32.11
13 (10)	0.03	1.13	2.90	4.48	6.13	7.54	9.51	11.59	13.67	15.83	18.07	19.98	21.59	22.78	23.79
MEAN	0.38	3.39	6.35	8.72	11.02	12.60	15.12	17.29	19.67	21.87	23.71	25.26	26.52	27.42	28.38
SD	0.18	1.49	2.29	3.06	3.66	4.01	4.61	4.99	5.54	6.05	6.51	6.69	6.78	6.80	6.96

APPENDIX E

Butylparaben – Human

Application amounts and rates

Skin Replicate	Activity applied (μCi)	Total BP (μg)	BP per area (μg/cm^2)
1	0.95	22.8	35.7
2	0.96	23.1	36.1
3	0.91	21.9	34.2
4	0.94	22.5	35.1
5	0.95	22.9	35.8
6	0.95	22.9	35.7
7	0.96	23.2	36.2
8	0.95	22.9	35.8
9	0.96	23.1	36.2
10	0.92	22.2	34.7
11	0.94	22.7	35.4
12	0.94	22.5	35.1
13	0.92	22.1	34.5
Mean	0.94	22.68	35.43
SD	0.02	0.42	0.66

Cumulative amount of radioactivity absorbed per area (µg equiv/cm^2)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10*	12*	14*	16	18	20	22	24
6 (1)	0.16	2.57	5.96	8.23	9.98	11.56	13.93	15.75	17.26	18.33	19.20	20.10	20.85	21.63	22.43
7 (2)	0.26	2.43	5.84	8.04	9.72	11.41	13.59	14.71	15.79	16.77	17.86	18.83	19.61	20.57	21.31
8 (3)	0.11	2.11	5.34	7.32	8.72	10.06	11.64	13.83	15.73	18.19	20.33	21.98	23.74	24.70	26.01
9 (4)	0.07	1.28	3.40	5.14	6.99	8.86	11.20	13.64	15.33	17.49	19.11	20.32	21.23	22.86	24.65
10 (5)	0.20	2.66	6.10	7.91	9.39	10.89	13.51	15.60	17.12	18.95	20.41	21.98	23.48	24.87	25.92
6 (6)	0.15	1.77	4.70	8.26	11.49	14.39	18.20	20.99	23.26	24.98	26.35	27.38	28.23	29.00	29.60
7 (7)	0.05	0.69	2.16	4.25	6.36	8.32	11.63	14.45	16.96	19.27	21.19	22.80	24.17	25.40	26.50
8 (8)	0.05	0.76	2.52	4.83	7.14	10.36	15.05	18.79	21.95	24.56	26.56	28.18	29.79	31.50	32.67
9 (9)	0.47	3.18	6.16	8.53	10.28	11.71	14.03	NA	NA	NA	22.08	23.68	25.17	26.65	27.88
10 (10)	0.40	3.08	5.99	8.30	10.16	11.68	14.10	NA	NA	NA	22.70	24.41	25.95	27.41	28.54
11 (11)	0.26	2.60	5.55	8.03	10.01	11.54	13.74	NA	NA	NA	19.64	20.83	21.88	22.89	23.73
12 (12)	0.07	1.07	2.64	4.14	5.45	6.45	8.14	NA	NA	NA	14.41	15.89	17.31	18.75	19.99
13 (13)	0.57	4.10	7.07	9.20	10.83	12.25	14.86	NA	NA	NA	23.51	25.28	26.87	28.14	29.20
MEAN	0.22	2.18	4.88	7.09	8.96	10.73	13.36	15.97	17.92	19.82	21.03	22.44	23.71	24.95	26.03
SD	0.17	1.02	1.64	1.80	1.88	1.99	2.38	2.60	3.00	3.16	3.33	3.41	3.54	3.61	3.60

*Fraction collector shuttle jammed following the 8-hour samples; 10-16 hour effluent from cells 9-13 collected in 16-hour vial.

Concentration-time course of total radioactivity (µg equiv/mL)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10*	12*	14*	16	18	20	22	24
6 (1)	0.06	0.98	1.38	0.92	0.71	0.64	0.48	0.37	0.30	0.22	0.17	0.18	0.15	0.16	0.16
7 (2)	0.11	0.88	1.38	0.89	0.68	0.69	0.44	0.23	0.22	0.20	0.22	0.20	0.16	0.19	0.15
8 (3)	0.04	0.81	1.31	0.80	0.56	0.54	0.32	0.44	0.38	0.50	0.43	0.33	0.35	0.19	0.26
9 (4)	0.03	0.49	0.86	0.70	0.75	0.76	0.47	0.49	0.34	0.44	0.33	0.25	0.18	0.33	0.36
10 (5)	0.08	1.00	1.39	0.74	0.60	0.61	0.53	0.42	0.31	0.37	0.30	0.32	0.30	0.28	0.21
6 (6)	0.06	0.66	1.18	1.44	1.31	1.17	0.77	0.56	0.46	0.35	0.28	0.21	0.17	0.15	0.12
7 (7)	0.02	0.26	0.60	0.85	0.86	0.79	0.67	0.57	0.51	0.47	0.39	0.33	0.28	0.25	0.22
8 (8)	0.02	0.29	0.71	0.94	0.93	1.31	0.95	0.75	0.64	0.53	0.40	0.33	0.32	0.35	0.23
9 (9)	0.19	1.10	1.21	0.96	0.71	0.58	0.47	NA	NA	NA	0.41	0.32	0.30	0.30	0.25
10 (10)	0.16	1.09	1.18	0.94	0.75	0.61	0.49	NA	NA	NA	0.43	0.35	0.31	0.29	0.23
11 (11)	0.10	0.95	1.20	1.00	0.80	0.62	0.44	NA	NA	NA	0.30	0.24	0.21	0.20	0.17
12 (12)	0.03	0.40	0.64	0.60	0.53	0.40	0.34	NA	NA	NA	0.32	0.30	0.29	0.29	0.25
13 (13)	0.23	1.43	1.20	0.86	0.66	0.57	0.53	NA	NA	NA	0.44	0.36	0.32	0.26	0.21
MEAN	0.09	0.79	1.09	0.90	0.76	0.72	0.53	0.48	0.39	0.38	0.34	0.28	0.26	0.25	0.22
SD	0.07	0.35	0.29	0.20	0.20	0.25	0.17	0.16	0.13	0.12	0.09	0.06	0.07	0.06	0.06

*Fraction collector shuttle jammed following the 8-hour samples; 10-16 hour effluent from cells 9-13 collected in 16-hour vial.

Cumulative percent of radioactivity absorbed

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10*	12*	14*	16	18	20	22	24
6 (1)	0.45	7.19	16.71	23.07	27.96	32.40	39.02	44.12	48.35	51.36	53.79	56.33	58.41	60.60	62.85
7 (2)	0.72	6.72	16.17	22.25	26.88	31.58	37.61	40.71	43.69	46.41	49.42	52.11	54.25	56.92	58.95
8 (3)	0.31	6.17	15.61	21.40	25.47	29.40	34.01	40.41	45.96	53.15	59.42	64.24	69.37	72.19	76.00
9 (4)	0.20	3.64	9.68	14.63	19.89	25.21	31.86	38.83	43.62	49.77	54.39	57.84	60.42	65.06	70.16
10 (5)	0.57	7.44	17.02	22.08	26.22	30.40	37.71	43.54	47.78	52.91	56.99	61.37	65.56	69.43	72.37
6 (6)	0.41	4.96	13.15	23.12	32.18	40.28	50.96	58.77	65.13	69.94	73.76	76.66	79.05	81.18	82.87
7 (7)	0.13	1.90	5.95	11.72	17.56	22.97	32.10	39.89	46.81	53.19	58.47	62.93	66.70	70.09	73.12
8 (8)	0.13	2.12	7.05	13.51	19.95	28.97	42.08	52.53	61.37	68.66	74.26	78.78	83.27	88.07	91.32
9 (9)	1.29	8.80	17.04	23.59	28.45	32.38	38.81	NA	NA	NA	61.07	65.49	69.63	73.70	77.10
10 (10)	1.15	8.87	17.24	23.91	29.27	33.64	40.60	NA	NA	NA	65.38	70.32	74.75	78.95	82.22
11 (11)	0.73	7.33	15.66	22.66	28.26	32.58	38.79	NA	NA	NA	55.42	58.78	61.76	64.60	66.98
12 (12)	0.21	3.05	7.53	11.77	15.52	18.35	23.18	NA	NA	NA	41.02	45.24	49.28	53.37	56.90
13 (13)	1.66	11.91	20.52	26.71	31.44	35.55	43.12	NA	NA	NA	68.25	73.36	77.97	81.67	84.76
MEAN	0.61	6.16	13.79	20.03	25.31	30.29	37.68	44.85	50.34	55.67	59.36	63.34	66.95	70.45	73.51
SD	0.49	2.94	4.68	5.15	5.35	5.62	6.63	7.10	8.21	8.72	9.40	9.70	10.11	10.30	10.34

*Fraction collector shuttle jammed following the 8-hour samples; 10-16 hour effluent from cells 9-13 collected in 16-hour vial.

Material Balance

Cell ID (replicate)	Receptor Fluid	Receptor wash	Skin Wash	Donor Chamber	Tape Strips	Skin	Total Recovery
6 (1)	62.85	0.78	18.35	0.15	0.85	6.34	89.33
7 (2)	58.95	0.48	24.57	0.36	0.54	4.05	88.95
8 (3)	76.00	0.70	11.15	0.56	0.88	7.72	97.00
9 (4)	70.16	1.08	10.55	0.16	0.56	6.05	88.58
10 (5)	72.37	0.66	6.30	0.24	0.69	5.84	86.10
6 (6)	82.87	0.48	14.34	0.30	0.75	7.07	105.81
7 (7)	73.12	1.08	9.61	0.10	0.70	11.29	95.89
8 (8)	91.32	0.70	7.65	0.49	0.62	7.59	108.37
9 (9)	77.10	0.91	11.32	0.06	0.86	5.98	96.23
10 (10)	82.22	0.49	15.40	1.14	2.37	6.69	108.32
11 (11)	66.98	0.46	26.42	2.01	2.84	6.10	104.81
12 (12)	56.90	0.84	34.63	1.47	3.06	9.12	106.03
13 (13)	84.76	0.70	13.22	0.79	3.55	6.15	109.16
MEAN	73.51	0.72	15.65	0.60	1.41	6.92	98.81
SD	10.34	0.21	8.29	0.60	1.11	1.77	8.65

Cell ID (replicate)	Absorbed	Absorbable	Unabsorbed
6 (1)	63.63	69.97	19.35
7 (2)	59.43	63.48	25.48
8 (3)	76.70	84.42	12.59
9 (4)	71.24	77.30	11.28
10 (5)	73.03	78.87	7.23
6 (6)	83.36	90.43	15.38
7 (7)	74.20	85.49	10.41
8 (8)	92.02	99.61	8.76
9 (9)	78.01	83.99	12.25
10 (10)	82.72	89.41	18.91
11 (11)	67.44	73.54	31.27
12 (12)	57.74	66.86	39.17
13 (13)	85.46	91.61	17.56
MEAN	74.23	81.15	17.66
SD	10.32	10.65	9.38

Cumulative amount of butylparaben absorbed per area (µg/cm^2)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10*	12*	14*	16	18	20	22	24
6 (1)	0.06	1.23	3.21	4.61	5.67	6.25	7.09	7.51	8.61	9.48	10.13	10.70	11.19	11.49	11.82
7 (2)	0.17	3.99	10.47	14.03	15.96	17.14	18.74	19.77	20.67	21.35	21.97	22.43	22.82	23.14	23.40
8 (3)	0.11	1.49	3.25	4.38	5.17	5.82	6.72	7.68	8.85	9.93	10.86	11.69	12.26	12.59	12.89
9 (4)	0.02	0.59	1.59	2.44	3.29	3.85	4.79	5.82	6.85	7.55	8.10	8.38	8.68	8.98	9.38
10 (5)	0.11	2.20	4.22	5.48	6.45	7.06	7.94	8.59	9.12	9.48	9.81	10.04	10.23	10.39	10.52
6 (6)	0.08	1.31	3.71	6.57	9.04	11.26	13.61	18.11	19.34	20.27	20.91	21.44	21.82	22.15	22.39
7 (7)	0.01	0.32	1.21	2.60	3.98	5.20	7.14	9.55	10.94	12.11	13.13	13.88	14.49	14.98	15.36
8 (8)	0.01	0.31	1.27	2.63	3.94	5.80	8.17	11.91	13.49	14.73	15.63	16.34	16.85	17.44	17.93
9 (9)	0.21	2.06	4.02	5.38	6.11	6.60	7.32	NA	NA	NA	9.33	9.77	10.20	10.57	10.86
10 (10)	0.13	1.73	2.68	4.34	5.05	5.47	6.05	NA	NA	NA	7.74	8.12	8.43	8.70	8.91
11 (11)	0.07	1.56	3.28	4.58	5.47	5.95	6.53	NA	NA	NA	7.72	7.92	8.12	8.29	8.43
12 (12)	0.00	0.42	1.13	1.73	2.22	2.47	2.82	NA	NA	NA	4.22	4.59	4.94	5.23	5.48
13 (13)	0.34	2.94	4.68	5.67	6.32	6.77	7.58	NA	NA	NA	10.16	10.72	11.18	11.52	11.52
MEAN	0.10	1.55	3.44	4.96	6.05	6.89	8.04	11.12	12.23	13.11	11.52	12.00	12.40	12.73	12.99
SD	0.10	1.08	2.43	3.09	3.42	3.67	4.02	5.16	5.18	5.21	5.19	5.26	5.30	5.35	5.38

*Fraction collector shuttle jammed following the 8-hour samples; 10-16 hour effluent from cells 9-13 collected in 16-hour vial.

Concentration-time course of butylparaben (µg equiv/mL)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10*	12*	14*	16	18	20	22	24
6 (1)	0.02	0.47	0.80	0.57	0.43	0.24	0.17	0.09	0.22	0.18	0.13	0.12	0.10	0.06	0.07
7 (2)	0.07	1.54	2.63	1.44	0.78	0.48	0.32	0.21	0.18	0.14	0.13	0.09	0.08	0.07	0.05
8 (3)	0.04	0.56	0.71	0.46	0.32	0.26	0.18	0.19	0.24	0.22	0.19	0.17	0.11	0.07	0.06
9 (4)	0.01	0.23	0.40	0.34	0.35	0.23	0.19	0.21	0.21	0.14	0.11	0.06	0.06	0.06	0.08
10 (5)	0.05	0.84	0.82	0.51	0.39	0.25	0.18	0.13	0.11	0.07	0.07	0.05	0.04	0.03	0.03
6 (6)	0.03	0.50	0.97	1.16	1.00	0.90	0.47	0.91	0.25	0.19	0.13	0.11	0.08	0.07	0.05
7 (7)	0.00	0.12	0.36	0.56	0.56	0.49	0.39	0.49	0.28	0.24	0.20	0.15	0.12	0.10	0.08
8 (8)	0.00	0.12	0.39	0.55	0.53	0.76	0.48	0.75	0.32	0.25	0.18	0.14	0.10	0.12	0.10
9 (9)	0.08	0.75	0.79	0.55	0.30	0.20	0.15	NA	NA	NA	0.10	0.09	0.09	0.07	0.06
10 (10)	0.05	0.65	0.39	0.67	0.29	0.17	0.12	NA	NA	NA	0.08	0.08	0.06	0.05	0.04
11 (11)	0.03	0.60	0.70	0.53	0.36	0.19	0.12	NA	NA	NA	0.06	0.04	0.04	0.03	0.03
12 (12)	0.00	0.17	0.29	0.25	0.20	0.10	0.07	NA	NA	NA	0.07	0.08	0.07	0.06	0.05
13 (13)	0.14	1.05	0.70	0.40	0.26	0.18	0.16	NA	NA	NA	0.13	0.11	0.09	0.07	0.00
MEAN	0.04	0.59	0.77	0.61	0.44	0.34	0.23	0.37	0.23	0.18	0.12	0.10	0.08	0.07	0.05
SD	0.04	0.41	0.60	0.33	0.23	0.25	0.14	0.31	0.06	0.06	0.05	0.04	0.03	0.02	0.03

*Fraction collector shuttle jammed following the 8-hour samples; 10-16 hour effluent from cells 9-13 collected in 16-hour vial.

Cumulative percent of butylparaben absorbed

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10*	12*	14*	16	18	20	22	24
6 (1)	0.16	3.44	9.00	12.90	15.88	17.51	19.87	21.05	24.13	26.56	28.38	29.99	31.36	32.21	33.11
7 (2)	0.48	11.03	28.98	38.82	44.16	47.43	51.84	54.71	57.18	59.07	60.78	62.06	63.13	64.03	64.75
8 (3)	0.31	4.35	9.50	12.80	15.12	17.00	19.65	22.43	25.87	29.01	31.74	34.17	35.82	36.79	37.67
9 (4)	0.05	1.68	4.51	6.93	9.37	10.95	13.63	16.56	19.50	21.49	23.06	23.85	24.69	25.57	26.70
10 (5)	0.31	6.13	11.78	15.30	18.00	19.70	22.16	23.98	25.47	26.47	27.39	28.02	28.56	29.01	29.37
6 (6)	0.22	3.67	10.38	18.40	25.30	31.53	38.10	50.71	54.14	56.76	58.54	60.02	61.08	62.01	62.69
7 (7)	0.03	0.87	3.33	7.17	11.00	14.35	19.70	26.35	30.20	33.43	36.22	38.29	39.99	41.34	42.40
8 (8)	0.03	0.88	3.56	7.36	11.01	16.23	22.84	33.28	37.71	41.19	43.71	45.68	47.11	48.76	50.11
9 (9)	0.58	5.70	11.11	14.87	16.90	18.25	20.24	NA	NA	NA	25.81	27.03	28.21	29.23	30.05
10 (10)	0.38	4.98	7.72	12.50	14.55	15.76	17.44	NA	NA	NA	22.28	23.39	24.29	25.07	25.68
11 (11)	0.20	4.40	9.25	12.93	15.45	16.79	18.42	NA	NA	NA	21.79	22.35	22.93	23.40	23.79
12 (12)	0.01	1.20	3.21	4.94	6.31	7.02	8.03	NA	NA	NA	12.00	13.08	14.06	14.89	15.60
13 (13)	0.99	8.54	13.58	16.45	18.34	19.64	22.01	NA	NA	NA	29.48	31.11	32.46	33.44	33.44
MEAN	0.29	4.38	9.69	13.95	17.03	19.40	22.61	31.13	34.27	36.75	32.40	33.77	34.90	35.83	36.57
SD	0.28	3.03	6.74	8.53	9.43	10.10	11.04	14.18	14.23	14.30	14.27	14.48	14.59	14.73	14.81

*Fraction collector shuttle jammed following the 8-hour samples; 10-16 hour effluent from cells 9-13 collected in 16-hour vial.

Cumulative amount of 4-hydroxybenzoic acid absorbed per area (µg/cm^2)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10*	12*	14*	16	18	20	22	24
6 (1)	0.09	0.58	1.22	1.83	2.42	2.86	3.54	4.59	5.18	5.54	6.14	6.53	6.88	7.14	7.44
7 (2)	0.06	0.54	1.11	1.58	2.01	2.33	2.78	3.14	3.56	4.06	4.44	4.73	4.94	5.21	5.45
8 (3)	0.08	0.58	1.10	1.59	2.04	2.43	3.31	3.83	4.48	5.27	6.00	6.56	6.93	7.21	7.50
9 (4)	0.06	0.40	0.89	1.39	1.93	2.34	2.99	3.70	4.31	4.86	5.25	5.58	5.87	6.30	6.70
10 (5)	0.05	0.29	0.58	0.81	1.04	1.18	1.43	1.64	1.91	2.21	2.45	2.58	2.72	2.85	2.92
6 (6)	0.08	0.53	1.10	1.85	2.62	3.28	4.35	5.77	6.63	7.32	7.94	8.38	8.77	9.05	9.34
7 (7)	0.02	0.29	0.68	1.25	1.74	2.24	3.12	4.03	4.75	5.53	6.12	6.69	7.22	7.76	8.21
8 (8)	0.02	0.28	0.77	1.39	1.98	2.77	4.06	5.67	6.70	7.66	8.38	9.02	9.76	10.58	11.05
9 (9)	0.24	1.08	2.05	2.91	3.51	4.06	5.03	NA	NA	NA	8.44	9.32	10.17	10.98	11.67
10 (10)	0.19	1.10	1.92	3.00	3.79	4.37	5.54	NA	NA	NA	9.50	10.48	11.26	12.11	12.88
11 (11)	0.16	0.90	1.74	2.57	3.24	3.74	4.65	NA	NA	NA	7.27	7.87	8.51	8.96	9.45
12 (12)	0.04	0.44	0.98	1.50	1.94	2.32	2.87	NA	NA	NA	5.47	6.25	6.91	7.68	8.33
13 (13)	0.20	1.07	1.88	2.60	3.23	3.70	4.47	NA	NA	NA	7.61	8.45	9.21	9.82	9.82
MEAN	0.10	0.62	1.23	1.87	2.42	2.89	3.70	4.05	4.69	5.31	6.54	7.11	7.63	8.13	8.52
SD	0.07	0.31	0.50	0.69	0.80	0.89	1.11	1.35	1.57	1.73	1.91	2.12	2.33	2.52	2.66

*Fraction collector shuttle jammed following the 8-hour samples; 10-16 hour effluent from cells 9-13 collected in 16-hour vial.

Concentration-time course of 4-hydroxybenzoic acid (µg equiv/mL)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10*	12*	14*	16	18	20	22	24
6 (1)	0.04	0.20	0.26	0.24	0.24	0.18	0.14	0.21	0.12	0.07	0.12	0.08	0.07	0.05	0.06
7 (2)	0.02	0.20	0.23	0.19	0.18	0.13	0.09	0.07	0.08	0.10	0.08	0.06	0.04	0.05	0.05
8 (3)	0.03	0.20	0.21	0.20	0.18	0.16	0.18	0.11	0.13	0.16	0.15	0.11	0.07	0.06	0.06
9 (4)	0.02	0.14	0.20	0.20	0.22	0.17	0.13	0.14	0.12	0.11	0.08	0.07	0.06	0.09	0.08
10 (5)	0.02	0.10	0.11	0.09	0.09	0.06	0.05	0.04	0.05	0.06	0.05	0.03	0.03	0.03	0.01
6 (6)	0.03	0.18	0.23	0.31	0.31	0.27	0.22	0.29	0.17	0.14	0.12	0.09	0.08	0.06	0.06
7 (7)	0.01	0.11	0.16	0.23	0.20	0.20	0.18	0.19	0.14	0.16	0.12	0.12	0.11	0.11	0.09
8 (8)	0.01	0.11	0.20	0.25	0.24	0.32	0.26	0.33	0.21	0.19	0.14	0.13	0.15	0.17	0.09
9 (9)	0.10	0.34	0.39	0.35	0.24	0.22	0.20	NA	NA	NA	0.17	0.18	0.17	0.16	0.14
10 (10)	0.08	0.36	0.33	0.44	0.32	0.24	0.24	NA	NA	NA	0.20	0.20	0.16	0.17	0.15
11 (11)	0.06	0.30	0.34	0.33	0.27	0.20	0.18	NA	NA	NA	0.13	0.12	0.13	0.09	0.10
12 (12)	0.02	0.16	0.22	0.21	0.18	0.15	0.11	NA	NA	NA	0.13	0.16	0.13	0.15	0.13
13 (13)	0.08	0.35	0.33	0.29	0.26	0.19	0.16	NA	NA	NA	0.16	0.17	0.15	0.12	0.00
MEAN	0.04	0.21	0.25	0.26	0.23	0.19	0.16	0.17	0.13	0.12	0.13	0.12	0.10	0.10	0.08
SD	0.03	0.10	0.08	0.09	0.06	0.06	0.06	0.10	0.05	0.05	0.04	0.05	0.05	0.05	0.05

*Fraction collector shuttle jammed following the 8-hour samples; 10-16 hour effluent from cells 9-13 collected in 16-hour vial.

Cumulative percent of 4-hydroxybenzoic acid absorbed

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10*	12*	14*	16	18	20	22	24
6 (1)	0.26	1.62	3.43	5.12	6.79	8.01	9.91	12.85	14.51	15.52	17.20	18.30	19.27	20.01	20.86
7 (2)	0.17	1.50	3.06	4.36	5.57	6.44	7.70	8.68	9.85	11.24	12.29	13.08	13.68	14.40	15.08
8 (3)	0.23	1.70	3.22	4.66	5.97	7.11	9.66	11.19	13.08	15.40	17.52	19.18	20.25	21.07	21.91
9 (4)	0.16	1.13	2.54	3.94	5.49	6.67	8.52	10.52	12.26	13.83	14.93	15.88	16.71	17.93	19.07
10 (5)	0.14	0.82	1.61	2.27	2.89	3.31	3.98	4.58	5.33	6.17	6.83	7.22	7.59	7.96	8.14
6 (6)	0.21	1.47	3.08	5.19	7.34	9.18	12.17	16.16	18.55	20.51	22.22	23.47	24.56	25.34	26.15
7 (7)	0.05	0.81	1.88	3.44	4.80	6.18	8.60	11.13	13.11	15.25	16.89	18.47	19.93	21.42	22.65
8 (8)	0.05	0.77	2.14	3.88	5.54	7.74	11.36	15.86	18.74	21.42	23.41	25.21	27.28	29.59	30.90
9 (9)	0.65	2.99	5.67	8.06	9.71	11.22	13.91	NA	NA	NA	23.35	25.77	28.12	30.38	32.27
10 (10)	0.56	3.16	5.52	8.64	10.91	12.59	15.94	NA	NA	NA	27.37	30.18	32.43	34.88	37.09
11 (11)	0.45	2.55	4.92	7.24	9.15	10.56	13.13	NA	NA	NA	20.53	22.20	24.02	25.30	26.68
12 (12)	0.12	1.26	2.78	4.27	5.51	6.59	8.17	NA	NA	NA	15.56	17.80	19.67	21.85	23.70
13 (13)	0.58	3.09	5.46	7.54	9.37	10.75	12.97	NA	NA	NA	22.08	24.53	26.72	28.51	28.51
MEAN	0.28	1.76	3.49	5.28	6.85	8.18	10.46	11.37	13.18	14.92	18.48	20.10	21.56	22.97	24.08
SD	0.21	0.89	1.43	1.97	2.31	2.56	3.19	3.77	4.39	4.86	5.47	6.06	6.64	7.20	7.59

*Fraction collector shuttle jammed following the 8-hour samples; 10-16 hour effluent from cells 9-13 collected in 16-hour vial.

APPENDIX F

Protocol and Amendments

Methylparaben and Butylparaben: *In Vitro* Dermal Penetration and Metabolism in Rat and Human Skin

Work Request Number 14807

Service Code 1377

Protocol

Haskell Animal Welfare Committee Number: BT133-GP

Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin DuPont-13966

TABLE OF CONTENTS

Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin DuPont-13966

INTRODUCTION

Methylparaben and butylparaben are alkyl esters of p-hydroxybenzoic acid and are used as antimicrobial agents in cosmetic and pharmaceutical formulations which may be applied to the skin. Given that skin contact can represent a major route of exposure, it is important to evaluate the penetration and metabolism of paraben esters following topical application.

OBJECTIVE

The objective of this study is to determine the penetration kinetics and metabolism of methylparaben and butylparaben in viable rat and human skin following a dermal exposure to a single, finite application of an oil-water emulsion formulation containing approximately 1% (w/v) of methylparaben and butylparaben prepared in separate formulations.

SPONSOR AND TEST FACILITY

This study is sponsored by the Cosmetic, Toiletry, and Fragrance Association (CTFA), Washington, DC. The sponsor's approval was effective the date the sponsor authorized the work on the contract.

The study will be conducted at Haskell Laboratory for Health and Environmental Sciences, E.I. du Pont de Nemours and Company, Newark, Delaware, USA.

REGULATORY COMPLIANCE

This study will be conducted in compliance with U.S. FDA (21 CFR 58) Good Laboratory Practice Standards, which are consistent with the OECD Principles of Good Laboratory Practice (as revised in 1997) published in ENV/MC/CHEM(98)17 and MAFF Japan Good Laboratory Practice Standards (59 NohSan Number 3850).[1-3]

This study has been designed to meet the testing requirements of the OECD Guideline for the Testing of Chemicals (Draft Guideline 428), Skin Absorption: *in vitro* Method (2002) and the EU Guidance Document on Dermal Absorption (2002).[4-5]

Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin DuPont-13966

MATERIALS AND METHODS

A. Test Substance(s)

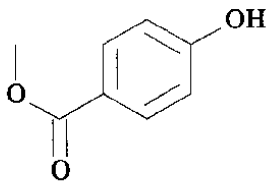
1. Methylparaben (CASN 99-76-3)

The test substance will be supplied by the sponsor and assigned a Haskell Laboratory Number upon receipt. Available information on the purity, composition, contaminants, synonyms, basic physical properties, hazards, and hazardous material classification(s) will be documented in the study records and/or report.

Molecular Weight: 152

Empirical Formula: $C_8H_8O_3$

Structure:



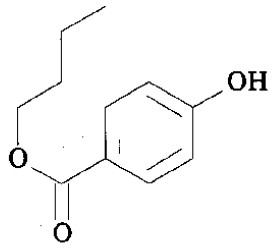
2. Butylparaben (CASN 94-26-8)

The test substance will be supplied by the sponsor and assigned a Haskell Laboratory Number upon receipt. Available information on the purity, composition, contaminants, synonyms, basic physical properties, hazards, and hazardous material classification(s) will be documented in the study records and/or report.

Molecular Weight: 194

Empirical Formula: $C_{11}H_{14}O_3$

Structure:

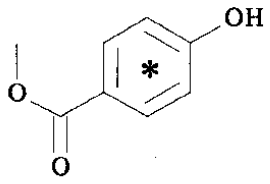


Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin DuPont-13966

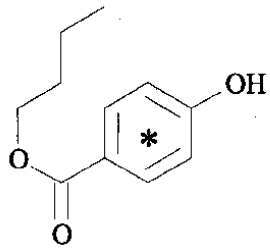
3. Radiolabeled Test Substances

The radiolabeled test substances will be supplied by the sponsor and assigned Haskell Laboratory Numbers upon receipt. Available information on the radio-chemical purity and specific activity will be documented in the study records and/or report.

[phenyl-^{14}C(U)methylparaben



[phenyl-^{14}C(U)butylparaben



*Denotes position of the radiolabel

4. Formulation Vehicle

The formulation vehicle will be supplied by the sponsor and assigned a Haskell Laboratory Number upon receipt. Available information on the purity, composition, contaminants, synonyms, basic physical properties, hazards, and hazardous material classification(s) will be documented in the study records and/or report.

B. Test System

1. Justification for Selection of Test System

Dermal contact is a route of human exposure.

The rat is being used in the *in vitro* test system as this species has been used in toxicological evaluations of methylparaben and butylparaben. Human skin is the comparative species of choice in order to aid in the extrapolation of *in vitro* data to the human *in vivo* situation.

In vitro dermal techniques employing diffusion cell systems have been shown to predict percutaneous absorption and metabolism of various chemicals *in vivo*.[6-8]

Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin DuPont-13966

2. Rat Skin

Male Wistar rats, approximately 6-8 weeks of age, will be supplied by Charles River Laboratories, Inc., Raleigh, North Carolina. Following a required quarantine period, rats will be removed from stock and uniquely identified by tail markings. Rats will be sacrificed by carbon dioxide asphyxiation, the fur from the dorsal region will be carefully shaved using clippers, and the skin excised. Skin specimens will be identified using the Haskell animal number.

3. Human Skin

Samples of human skin from local surgeons or from the International Institute for the Advancement of Medicine (IIAM) will be obtained fresh. The source and identity of the skin sample (sex, anatomical locale, and approximate age of donor) will be documented in the study records. Skin specimens will be identified using a unique code (e.g., HCFT-26A = Human, Caucasian, Female, Thigh sample 26-A).

Skin specimens will be stored in a physiologic buffer (i.e., Hepes-buffered Hanks' balanced salt solution) and maintained on ice or refrigerated at 0-10°C until prepared for use and should be used within 12 hours of removal from the donor.

C. Dose Information

1. Dose Formulation

Radiolabeled and non-radiolabeled methylparaben and butylparaben will be blended with the formulation vehicle and deionized (DI) water to produce an oil-water emulsion. The formulation methodology used will be documented in the study records and presented in the final report. A summary of the formulations, target concentrations and skin doses are presented in the table below.

Formulation	Target Concentration	Target Skin Dose
1% (w/v) methylparaben emulsion	10 g methylparaben/L	100 μg methylparaben/cm^2
1% (w/v) butylparaben emulsion	10 g butylparaben/L	100 μg butylparaben/cm^2

2. Homogeneity, Concentration, and Stability

The homogeneity and amount of radiolabeled methylparaben and butylparaben (μCi/g) in each formulation will be determined by subjecting aliquots of the prepared formulation to radioanalysis by liquid scintillation counting (LSC).

The concentration of methylparaben and butylparaben in each formulation will be determined chromatographically using a suitable analytical method, in lieu of calculated concentration values based on weights of formulation ingredients.

Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin DuPont-13966

The stability of radiolabeled methylparaben and butylparaben will be determined using a suitable analytical method.

The analytical methods used will be documented in the study records and presented in the final report.

The results of the homogeneity and concentration analyses will be used to calculate the specific activity of radiolabeled methylparaben and butylparaben (μCi/mg) for each formulation.

3. Dose Administration

The emulsions will be applied as a single finite dose with a target application rate of 10 μL/cm^2. The *in vitro* cells have an exposure area of 0.64 cm^2, which requires a dose of 6.4 μL.

4. Dose Groups

This study will be composed of the following dose groups and target parameters.

a. 1% (w/v) emulsion of methylparaben (10 g methylparaben/L)

Species	Group	Dose Concentration (g methylparaben/L)	Skin Dose Level (μg methylparaben/cm^2)	Number of Skin Preparations	μCi/skin
Rat	A	10	100	10	1.0
Human	B	10	100	10	1.0

b. 1% (w/v) emulsion of butylparaben (10 g butylparaben/L)

Species	Group	Dose Concentration (g butylparaben/L)	Skin Dose Level (μg butylparaben/cm^2)	Number of Skin Preparations	μCi/skin
Rat	C	10	100	10	1.0
Human	D	10	100	10	1.0

D. Preparation of Skin Membranes

Samples of rat and human skin will be dermatomed to approximately 450 μm using a Padgett Electro Dermatome® (Padgett Instruments, Inc., Kansas City, MO). The target number of replicate preparations per species should be represented by at least three donors.

E. *In Vitro* Diffusion Cells

An automated flow-through diffusion cell system (PermeGear, Inc., Bethlehem, PA) with an exposure area of 0.64 cm^2 and a receptor chamber volume of approximately 250 μL will be used for this study. The skin membrane will be placed, stratum corneum uppermost, on to the top of

Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin DuPont-13966

the receptor chamber. The donor (top) chamber will then be placed over the skin section and clamped in-place. A recirculating water bath will be used to maintain a skin temperature of approximately 32°C.

F. *In Vitro* Percutaneous Absorption and Metabolism of Methylparaben and Butylparaben

1. Pre-Dose Procedures

a. Flow-Through System Equilibration

The flow-through diffusion cells will be perfused at approximately 1.5 mL/h with physiologic buffer (e.g., Hepes-buffered Hanks' balanced salt solution) containing an antibiotic (e.g., gentamicin) and surfactant (e.g., bovine serum albumin). Prior to application of the test emulsion the system will be equilibrated for approximately 30 minutes.

b. Assessment of Membrane Integrity

The integrity of each membrane will be assessed by measurement of electrical resistance following equilibration and prior to application of test formulation.[9]

2. Application of the Formulated Test Substance

The prepared formulations will be applied to the skin surface, via the donor chamber, as a single finite dose distributed evenly over the exposure area at a rate of 10 $\mu L/cm^2$.

The donor chamber will remain unoccluded for the duration of the exposure period.

3. Dose Determination

The actual amount of radioactivity administered to the skin will be measured by counting replicate mock doses and using the mean value as the amount of radioactivity applied. The amount of paraben ester applied will be based on the total radioactivity determination and the verified specific activity of the formulated emulsion.

4. Exposure Period

The exposure period will be 24 hours for all skin preparations.

5. Serial Sampling of Receptor Fluid

Following dose application, samples of receptor fluid will be automatically collected directly into glass vials. The frequency and number of collections will be documented in the study records and presented in the final report.

Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin DuPont-13966

G. Terminal Procedures

1. Washing of the Application Skin Site

All skin preparations will be washed at the conclusion of the 24-hour exposure using at least 3 x 1 mL of a 2% soap solution (e.g., Ivory® Soap) followed by at least 1 x 1 mL rinse with DI water. The wash will be collected into a liquid scintillation vial.

The donor chamber will be removed and rinsed with approximately 2 mL of methanol directly into a liquid scintillation vial.

The skin membrane will be removed from the receptor chamber and tape-stripped using Leukotape® P (Beiersdorf, Hamburg, Germany). The tapes will be placed into a glass vial and extracted with methanol. The remaining skin piece will be placed into a glass scintillation vial for digestion.

2. Sample Handling and Processing

Samples not immediately processed for analysis will be stored frozen at ≤-10°C (i.e., serial receptor fluid and application skin samples) or refrigerated at 0-10°C (i.e., skin wash, tape strips, and donor chamber rinse).

Each skin piece will be digested using Soluene®-350. Heating at approximately 60°C accompanied by constant shaking may be used to facilitate sample digestion.

H. Determination of Radioactivity

Liquid scintillation cocktail (e.g., Ultima Gold™ XR) will be added directly to vials containing aliquots of serial receptor fluid samples, and to the entire vial contents of the donor chamber rinse, skin wash, and tape strips extracts.

Hionic-Fluor™ will be added to vials containing the digested skin piece.

The samples will be analyzed by liquid scintillation counting (LSC) for total radioactivity.

I. Liquid Scintillation Counting

Samples will be analyzed in a Packard liquid scintillation counter. Samples will be counted for 10 minutes or until 160,000 disintegrations are accumulated (0.5%, 2σ), whichever comes first.

The limit of detection (LOD) for the analysis of each sample will be taken as twice the background disintegration rate obtained from analysis of appropriate blank samples.

Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin DuPont-13966

J. Analysis of Receptor Fluid Samples

Aliquots of serial receptor fluid samples will be analyzed for paraben esters and 4-hydroxybenzoic acid using a suitable analytical method, which will be documented in the study records and presented in the final report. Receptor fluid samples will also be screened for other potential phase II metabolites and the methods and results presented in the final report.

K. Statistical Analyses and Data Presentation

Group data will be represented at Mean ± SD. Where appropriate, statistical significance will be assessed by the Student's t-test. Other statistical evaluations may be performed, if necessary.

Total recovery of the applied radioactivity will be a sum of the receptor fluid samples, amount washed from the skin, amount in tape strips, and the amount in/on the skin not removed by washing, which should result in a group mean material balance of 100% ± 10%.

The cumulative amount of radioactivity, methylparaben, butylparaben, and 4-hydroxybenzoic acid detected in the receptor fluid at each serial collection time-point will be plotted against time (in hours) to produce an absorption profile.

L. Pilot Experiments

Pilot experiments may be conducted to establish definitive methods and procedures. Results of the pilot experiments will not be included in the final report but will be maintained with the study records.

SAFETY AND HOUSEKEEPING

All chemicals used during this study will be handled according to the procedures specified in the MSDS and disposed of according to the Stine-Haskell Waste Disposal Guidelines and the area Safety, Health and Environmental (SHE) manual. The storage, handling, and disposal of human skin used in the conduct of this study will comply with those procedures outlined in the area SHE manual.

RECORDS AND SAMPLE STORAGE

All data and records for analytical characterizations conducted by the sponsor will be retained by the sponsor. Raw data and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware for a period of 6 months without additional cost to the sponsor. At the end of the 6-month period, Haskell will return the records to the sponsor.

Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin DuPont-13966

PROPOSED STUDY DATES

Date of Approval:	Date Study Director signed Protocol
Experimental Start:	February 2004
Experimental Termination:	June 2004
Study Completion:	November 2004

REFERENCES

1. U.S. FDA Good Laboratory Practice Standard (21 CFR 58). (1989)
2. OECD Principles of Good Laboratory Practice (as revised in 1997, published in ENV/MC/CHEM(98)17 (OCDE/GD(92)32). (1987)
3. MAFF Japan Good Laboratory Practice Standards (59 NOHSan No. 3850). (1985)
4. OECD Guideline for the Testing of Chemicals (Draft Guideline 428), Skin Absorption: *in vitro* Method (2002).
5. EU Guidance Document on Dermal Absorption (Draft Revision 5). (2002)
6. Scott RC, Batten PL, Clowes HM, Jones BK and Ramsey JD (1992). Further Validation of an *In Vitro* Method to Reduce the Need for *In Vivo* Studies for Measuring the Absorption of Chemicals through Rat Skin. Fundamental and Applied Toxicology 19, 484-492.
7. Ramsey JD, Woollen BH, Auton TR and Scott RC (1994). The Predictive Accuracy of *In Vitro* Measurements for Dermal Absorption of a Lipophilic Penetrant (Fluazifop-Butyl) through Rat and Human Skin. Fundamental and Applied Toxicology 23, 230-236.
8. Scott RC, Walker M and Dugard PH (1986). A comparison of the *in vitro* permeability properties of human and some laboratory animal skins. International Journal of Cosmetic Science 8, 189-194.
9. Fasano WJ, Manning LA and Green JW (2002). Rapid Integrity Assessment of Rat and Human Epidermal Membranes for *In Vitro* Dermal Regulatory Testing: Correlation of Electrical Resistance with Tritiated Permeability. Toxicology In Vitro 16, 731-740.

Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin DuPont-13966

SIGNATURES

Approved by: _____ 9-DEC-2003
William J. Pisano, Sr., B.S. Date
Study Director

Sponsor Representative: Linda Lorentz December 10, 2003
Linda Lorentz Date
CTFA Representative

Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin DuPont-13966

Work Request Number 14807
Service Code 1377
Protocol Amendment 1

The protocol is amended as follows:

1. Page 6, C. Dose Information, 1. Dose Formulation, replace table with the following:

Formulation	Target Concentration	Target Skin Dose
0.8% (w/v) methylparaben emulsion	8 g methylparaben/L	80 µg methylparaben/cm^2
0.4% (w/v) butylparaben emulsion	4 g butylparaben /L	40 µg butylparaben /cm^2

Rationale: Sponsor request.

2. Page 7, 4. Dose Groups, a. 1% (w/v) emulsion of methylparaben (10 g methylparaben/L) with the following:

a. 0.8% (w/v) emulsion of methylparaben (8 g methylparaben/L)

Species	Group	Dose concentration (g methylparaben/L)	Skin dose level (µg methylparaben/cm^2)	Number of skin Preparations	Target µCi/skin
Rat	A	8	80	10	1.0
Human	B	8	80	10	1.0

Rationale: Sponsor request.

Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin DuPont-13966

3. Page 7, 4. Dose Groups, b. 1% (w/v) emulsion of butylparaben (10 g butylparaben/L) with the following:

b. 0.4% (w/v) emulsion of butylparaben (4 g butylparaben/L)

Species	Group	Dose concentration (g butylparaben/L)	Skin dose level (μg butylparaben/cm^2)	Number of skin Preparations	Target μCi/skin
Rat	C	4	40	10	1.0
Human	D	4	40	10	1.0

Rationale: Sponsor request.

Approved by: _____ 16-FEB-2004
William J. Pearce, B.S.
Study Director
Date

Sponsor Representative: _____ 2-20-04
Linda Lorenz
CTFA Representative
Date

Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin DuPont-13966

Work Request Number 14807
Service Code 1377
Protocol Amendment 2

The protocol is amended as follows:

1. Page 3, Regulatory Compliance, replace first paragraph with the following:

   This study will be conducted in compliance with U.S. EPA FDA (21 CFR part 58) Good Laboratory Practice Standards, which are compatible with the OECD Principles of Good Laboratory Practice (as revised 1997), ENV/MC/CHEM(98)17, OECD, Paris, 1998, and MAFF Japan Good Laboratory Practice Standards (11 NohSan Number 6283).

Rationale: Corrected to reflect current nomenclature.

2. Page 3, Regulatory Compliance, replace second paragraph with the following:

   This study has been designed to meet the testing requirements of the OECD Guideline for the Testing of Chemicals (Draft Guideline 428), Skin Absorption: *in vitro* Method (2002), and OECD Draft Guidance Document for the Conduct of Skin Absorption Studies (2002).[4-5]

Rationale: Corrected reference for OECD guidance document.

3. Page 4, A. Test Substance, A. Methylparaben, replace first sentence with the following:

   The test substance was supplied by the sponsor and assigned Haskell Laboratory Number 26201 upon receipt.

Rationale: Added Haskell number.

4. Page 4, A. Test Substance, B. Butylparaben, replace first sentence with the following:

   The test substance was supplied by the sponsor and assigned Haskell Laboratory Number 26202 upon receipt.

Rationale: Added Haskell number.

5. Page 5, 3. Radiolabeled Test Substances, replace first sentence with the following:

   The radiolabeled test substances were supplied by the sponsor and assigned Haskell Laboratory Numbers 22705-84 (methylparaben) and 22705-85 (butylparaben) upon receipt.

Rationale: Added Haskell numbers.

Methylparaben and Butylparaben:
In Vitro Dermal Penetration and Metabolism in Rat and Human Skin DuPont-13966

6. Page 5, 4. Formulation Vehicle, replace first sentence with the following:

   The formulation vehicles were supplied by the sponsor and assigned Haskell Laboratory Numbers 26324 (Phase A), 26325 (Phase B), and 26326 (Phase C) upon receipt.

Rationale: Added Haskell numbers.

7. Page 10, Records and Sample Storage, replace paragraph with the following:

   All data and records for analytical characterizations conducted by the sponsor will be retained by the sponsor. Raw data and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware, and will be returned to the sponsor within 6 months after the final report issues.

Rationale: Corrected to reflect current nomenclature.

8. Page 11, delete references 1-3.

Rationale: References defined in Regulatory Compliance section.

9. Page 11, replace reference 5 with the following:

   OECD Draft Guidance Document for the Conduct of Skin Absorption Studies. OECD Environmental Health and Safety Publication Series on Testing and Assessment No. 28 (2002).

Rationale: Corrected reference for OECD guidance document.

Approved by:  22-Sept-2004

William J. Fasano, B.S.
Study Director

Date

TRADE SECRET

Study Title

Butylparaben:
In Vitro Dermal Penetration and Metabolism Using Full Thickness Human Skin

TEST GUIDELINES: OECD Guideline for the Testing of Chemicals. Draft New Guideline 428: Skin Absorption: *in vitro* Method (2002)

OECD Draft Guidance Document for the Conduct of Skin Absorption Studies. OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28 (2002)

AUTHOR: William J. Fasano, Sr., B.S.

STUDY COMPLETED ON: November 17, 2004

PERFORMING LABORATORY: E.I. du Pont de Nemours and Company
Haskell[SM] Laboratory for Health and Environmental Sciences
Elkton Road, P.O. Box 50
Newark, Delaware 19714-0050

LABORATORY PROJECT ID: DuPont-15565

WORK REQUEST NUMBER: 15475

SERVICE CODE NUMBER: 1377

SPONSOR: Cosmetic, Toiletry, and Fragrance Association (CTFA)
Washington, DC
U.S.A.

CERTIFICATION

I, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Issued by Study Director: _____ 17-Nov-2004

William J. Fasano, Sr., B.S.
Research Toxicologist

Date

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STUDY INFORMATION

Substance Tested:	Butylparaben
Synonyms/Codes:	• Butyl Paraben • B3140 (Lot No.)
CAS Registry Number:	94-26-8
Physical Characteristics:	White crystalline powder
Stability:	The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.
Study Initiated/Completed:	July 15, 2004 / (see report cover page)
Experimental Start/Completion:	July 15, 2004 / July 20, 2004

SUMMARY

The penetration kinetics and first-pass metabolism of butylparaben using viable, full-thickness human skin has been determined. The active ingredient was formulated as an oil-in-water emulsion at a target concentration of 0.4%. Samples of fresh full-thickness human skin were mounted *stratum corneum* uppermost, onto a flow-through diffusion cell system with an exposure area of 0.64 cm^2. The underside of each skin specimen was perfused with sterile-filtered Hepes-buffered Hanks' balanced salt solution containing gentimicin (0.05 mg/mL) and bovine serum albumin (3.75%). The formulated emulsion was applied as a finite dose at a rate of 10 $\mu L/cm^2$ (n = 6 replicates). Penetration was followed using [^{14}C]-labeled active ingredient, which was uniformly blended into the emulsion. The amount of active applied per area of skin was approximately 31.9 $\mu g/cm^2$. The applied formulation remained in contact with the skins for 24 hours without occlusion. During the 24-hour exposure period, serial receptor fluid samples were collected hourly for the first 6 hours and then every other hour until termination. At the end of the exposure period, the skin surface was washed with a dilute soap solution to remove excess formulation and then tape-stripped to remove the *stratum corneum.* Distribution of the applied radiolabeled material was determined by liquid scintillation counting. First-pass metabolism was determined by quantitative analysis of serial receptor fluid samples for butylparaben and 4-hydroxybenzoic acid using HPLC-mass spectrometry.

Following application of a 0.4% butylparaben emulsion to viable, full-thickness human skin, total penetration at 24 hours post-exposure was 21.01%. The principle metabolite, 4-hydroxybenzoic acid, was detected in the receptor fluid over the course of the exposure phase with barely detectable levels of unmetabolized butylparaben in receptor fluid from only one of the six skin replicates.

Overall these data suggest that the dermal bioavailability of butylparaben from an oil-in-water emulsion was low and that first pass metabolism resulted in complete hydrolysis to the primary acid metabolite.

INTRODUCTION

Butylparaben is an alkyl ester of p-hydroxybenzoic acid and is used as an antimicrobial agent in cosmetic and pharmaceutical formulations which may be applied to the skin. Given that skin contact can represent a major route of exposure, it is important to evaluate the penetration and metabolism of butylparaben following topical application.

The objective of this experiment was to determine the penetration rate and first-pass metabolism of butylparaben from an oil-in-water emulsion using viable, full-thickness human skin mounted in a flow-through cell diffusion system.

MATERIALS AND METHODS

A. Test Guidelines

The study design complies with the following test guidelines:

- OECD Guideline for the Testing of Chemicals. Draft New Guideline 428: Skin Absorption: *in vitro* Method (2002).
- OECD Draft Guidance Document for the Conduct of Skin Absorption Studies. OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28 (2002).

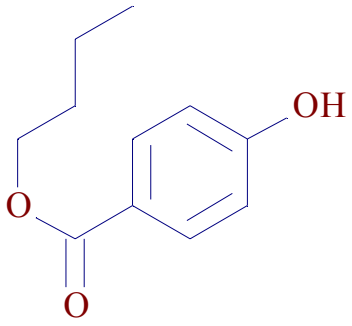
B. Test Substances

1. Butylparaben (CASN 94-26-8)

The test substance was supplied by Protameen Chemicals, Inc., assigned Haskell Laboratory Number 26202 upon receipt, and stored at room temperature.

Molecular Weight:	194
Empirical Formula:	$C_{11}H_{14}O_3$
Lot Number:	B3140
Purity:	99.5%
Octanol-water partition coefficient (log P):	3.46

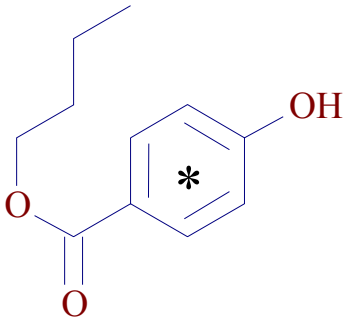
Structure:



2. Radiolabeled Test Substance

The radiolabeled test substance was supplied by Amersham Biosciences U.K. Limited, and assigned Haskell Laboratory Number 22705-85 upon receipt.

[phenyl-^{14}C(U)butylparaben



*Denotes position of the radiolabel

Code: CFQ13766

Batch: 1

Specific Activity: 75 µCi/mg

Purity: 99.2%

3. Formulation Vehicle

The formulation vehicles required to prepare the oil-in-water emulsions were supplied by Cosmetech Laboratories, Inc., Fairfield, New Jersey, and were assigned Haskell Laboratory Numbers 26324 (Phase A), 26325 (Phase B), and 26326 (Phase C) upon receipt. The vehicles were stored at room temperature.

C. Test System

Dermal contact is a route of human exposure.

In vitro dermal techniques employing diffusion cell systems have been shown to predict percutaneous absorption of various chemicals *in vivo*.[1-3] Additional guidance has been provided for cosmetic ingredients.[4]

Samples of human skin from local surgeons were obtained fresh. The source and identity of the skin sample (sex, anatomical locale, and approximate age of donor) were documented in the study records. Skin specimens were identified using a unique code (e.g., HCFA-26A = Human, Caucasian, Female, Abdomen sample 26-A).

At the time of harvest, skin specimens were stored in Hepes-buffered Hanks' balanced salt solution (containing 0.05 mg/L gentimicin) and were maintained on ice until prepared for use. All skin specimens were used in less than 4 hours of removal from donors.

D. Dose Information

1. Dose Formulation

The oil-in-water emulsion was prepared by heating Phase A and B to approximately 75°C. Radiolabeled and non-radiolabeled butylparaben was then added to Phase A and mixed. Phase B was then added to Phase A containing the butylparaben active ingredient, followed by the addition of Phase C. The emulsion was mixed, cooled, and stored refrigerated at 0-10°C. A summary of the target parameters is presented in the table below.

Formulation	Target Concentration	Target Skin Dose
0.4% (w/v) butylparaben emulsion	4 g butylparaben /L	40 μg butylparaben /cm^2

2. Homogeneity, Concentration, and Stability

The homogeneity and amount of radiolabeled butylparaben (μCi/g) in the formulation was determined by subjecting aliquots of the prepared formulation to radioanalysis by liquid scintillation counting (LSC).

The concentration of butylparaben in the formulation was determined chromatographically using the following analytical equipment and method:

System: Agilent 1100 Series Equipment (Agilent Technologies, Palo Alto, CA, USA)
Column: Zorbax SB-C18 4.6 mm x 75 mm, 3.5 µm particles
Column temperature: Ambient
Mobile phases: A: 0.5% trifluoroacetic acid in water
B: Acetonitrile

Gradient:

Time (min)	%A	%B
0.00	90	10
6.00	0	100
7.00	0	100
7.01	90	10

Flow rate: 1 mL/min
UV Wavelength 258 nm

The results of the homogeneity and concentration analyses were used to calculate the specific activity of radiolabeled butylparaben (µCi/mg).

The purity of the neat radiolabeled butylparaben and the stability of the radiolabeled butylparaben in the prepared emulsion were determined using the following analytical equipment and method:

System: Agilent 1100 Series Equipment (Agilent Technologies, Palo Alto, CA, USA)
Column: Zorbax SB-C18 4.6 mm x 150 mm, 3.5 µm particles
Column temperature: Ambient
Mobile phases: A: 2 mM ammonium acetate in water
B: Acetonitrile

Gradient:

Time (min)	%A	%B
0.00	90	10
27.00	40	60
30.00	0	100
30.01	90	10

Flow rate: 1 mL/min
Radiodetection:
- Radiomatic™ Series 500TR Flow Scintillation System, 31.9 µL CaF2 solid cell (neat radiolabeled materials)
- Fraction collection (Foxy 200™, Isco, Inc., Lincoln, NE) followed by liquid scintillation counting (prepared emulsion)

3. Dose Groups

This study was composed of the following dose group and target parameters.

a. 0.4% (w/v) emulsion of butylparaben (4 g butylparaben/L)

Species	Group	Dose Concentration (g butylparaben/L)	Skin Dose Level (µg butylparaben/cm^2)	Number of Skin Preparations	µCi/skin
Human	A	4	40	6	1.0

E. Preparation of Full-Thickness Skin

Samples of human abdominal skin were cleaned of subcutaneous fat. No additional processing was performed.

F. *In Vitro* Diffusion Cells

An automated flow-through diffusion cell system (PermeGear, Inc., Bethlehem, PA) with an exposure area of 0.64 cm^2 and a receptor chamber volume of approximately 250 µL was used for this study. The skin membrane was placed, *stratum corneum* uppermost, on to the top of the receptor chamber. The donor (top) chamber was then placed over the skin section and clamped in-place. A recirculating water bath was used to maintain a skin temperature of approximately 32°C.

G. *In Vitro* Percutaneous Absorption and Metabolism of Butylparaben

1. Pre-Dose Procedures

a. Flow-Through System Equilibration

The flow-through diffusion cells were perfused at approximately 1.5 mL/h with 25 mM Hepes-buffered Hanks' balanced salt solution containing 0.05 mg/mL gentamicin and 3.75% bovine serum albumin. Prior to application of the test emulsion the system was equilibrated for approximately 30 minutes.

b. Assessment of Membrane Integrity

The integrity of each membrane was assessed by measurement of electrical impedance following equilibration and prior to application of test formulation.[5-6] Membranes with an impedance of ≥ 20.2 k-ohms were considered intact and retained for use.

2. Application of the Formulated Test Substance

The prepared formulation was applied to the skin surface, via the donor chamber, as a single finite dose at a rate of 10 µL/cm^2. The dose (6.4 µL) was distributed evenly over the skin surface using a spreader device.

The donor chamber remained unoccluded for the duration of the exposure period.

3. Dose Determination

The actual amount of radioactivity administered to the skin was measured by counting replicate mock doses and using the mean value as the amount of radioactivity applied. The amount of butylparaben applied was based on the total radioactivity determination and the verified specific activity of the formulated emulsion.

4. Exposure Period

The exposure period was 24 hours for all skin preparations.

5. Serial Sampling of Receptor Fluid

Following dose application, samples of receptor fluid were collected at 1, 2, 3, 4, 5, 6 hours, and every other hour until 24 hours.

H. Terminal Procedures

1. Washing of the Application Skin Site

All skin preparations were washed at the conclusion of the 24-hour exposure using at least 3 x 1 mL of a 2% soap solution (e.g., Ivory® Soap) followed by at least 1 x 1 mL rinse with DI water. The wash was collected into a liquid scintillation vial.

The donor chamber was removed and rinsed with approximately 2 mL of acetonitrile directly into a liquid scintillation vial.

The skin membrane was removed from the receptor chamber and tape-stripped 5 times using Leukotape® P (Beiersdorf, Hamburg, Germany). The tapes were placed into a glass vial and extracted with acetonitrile. The remaining skin piece was placed into a glass scintillation vial and digested using Soluene®-350.

I. Determination of Radioactivity

Liquid scintillation cocktail (e.g., Ultima Gold™ XR) was added directly to vials containing aliquots of serial receptor fluid samples, and to the entire vial contents of the donor chamber rinse, skin wash, and tape strip extracts.

Hionic-Fluor™ was added to vials containing the digested skin piece.

The samples were analyzed by liquid scintillation counting (LSC) for total radioactivity.

J. Liquid Scintillation Counting

Samples were analyzed in a Packard liquid scintillation counter. Samples were counted for 10 minutes or until 160,000 disintegrations were accumulated (0.5%, 2σ), whichever came first.

The limit of detection (LOD) for the analysis of each sample was taken as twice the background disintegration rate obtained from analysis of appropriate blank samples.

K. Analysis of Receptor Fluid Samples

Samples of receptor fluid along with reference standards, were mixed with acetonitrile filtered and analyzed using a Waters Alliance® 2795 Liquid Chromatograph (LC) coupled to a Micromass Quattro micro™ tandem quadrupole mass spectrometer (MS) equipped with a MASSLYNX data acquisition system (Micromass Inc., Manchester, UK).

HPLC Conditions:

Column:	Agilent Zorbax SB-C18, 2.1 x 30 mm, 3.5 µm particles
Column temperature:	Ambient
Injection volume:	5 µL
Solvent:	A: 0.5% formic acid in water B: Acetonitrile

Gradient:

Time (min)	A	B
0	97	3
4.00	0	100
7.00	0	100
7.01	97	3

Flow rate:	0.30 mL/min
Run time::	10 minutes

MS Conditions:

Ionization mode:	Electrospray negative (ES-)
Capillary voltage:	3.20 kV
Cone voltage	(a) 21 V for 4HBA (b) 31 V for BP
Extractor voltage:	2.0 V
Source temperature:	140°C
Cone temperature:	Not controlled
Desolvation temperature:	350°C
Cone gas flow (approx.):	100 L/Hr (nitrogen)
Desolvation gas flow (approx.):	650 L/Hr (nitrogen)
Collision energy:	(a) 13 eV for 4HBA (b) 22 eV for BP
Collision gas::	Argon
Mode:	Multiple Reaction Monitoring (MRM) (a) 137.03 > 92.78 for 4HBA (b) 139.03 > 94.78 for ^{14}C-4HBA (c) 193.20 > 91.90 for BP (d) 195.20 > 93.90 for ^{14}C-BP

L. Statistical Analyses and Data Presentation

Group data is represented as Mean ± SD.

RESULTS AND DISCUSSION

A. Purity of Radiolabeled Butylparaben (Figure 1, Appendix A)

The radiochemical purity of the neat [^{14}C]-butylparaben (Amersham Biosciences UK Limited) was determined to be greater than 99%. A representative radiochromatogram is presented in Figure 1.

B. Storage Stability of [^{14}C]-Active Ingredient in Emulsions (Figure 2, Appendix A)

When incorporated into the oil-in-water emulsion, and stored refrigerated 0-10°C, the radiolabeled active ingredient was stable (>99%) for approximately 3 months (Figure 2). Therefore, the formulated emulsion was stable under the conditions used in this study.

Documents relating to the formulation ingredients and procedures for preparation of the emulsion from Cosmetech Inc., along with certificates of analysis (COA) for non-radiolabeled butylparaben and 4-hydroxybenzoic acid reference material, are presented in Appendix A.

C. Verified Concentrations for the Emulsions

The HPLC-UV verified chemical concentration for the 0.4% butylparaben emulsion (0.41%) was comparable to the target concentration.

D. 0.4% Butylparaben (Tables 1-5, Figures 3-7, Appendix B)

Mean data for the total radioactivity applied (µCi), the total amount applied (µg), and the application rate (µg/cm²) for butylparaben are presented in Table 1.

Key observations of mean data:

- The cumulative amount of radioactivity, butylparaben, and 4-hydroxybenzoic acid absorbed per area at 24 hours was 6.74 µg equiv/cm^2, < 0.22 µg/ cm^2, and 7.69 µg/ cm^2, respectively (Table 2, Figure 3); butylparaben was detected in only 1 of 6 cells.

- Based on the concentration-time course data, the peak for total radioactivity (0.10 µg equiv/mL), butylparaben (0.006 µg/mL), and 4-hydroxybenzoic acid (0.13 µg/mL) occurred at 18-20 hours, 12 hours, and 12 hours, respectively (Table 3, Figure 4).

- The cumulative amount absorbed at 24 hours for total radioactivity, butylparaben, and 4-hydroxybenzoic acid was 21.01%, < 0.67%, and 24.00%, respectively (Table 4, Figure 5);

of the total radioactivity absorbed, butylparaben and 4-hydroxybenzoic acid represented < 3.19% and 114.1%, respectively.

- At the end of the exposure phase, the total amount of radioactivity absorbed (receptor fluid only), the absorbable dose (absorbed plus tape-stripped skin), and the unabsorbed dose (wash, donor chamber rinse, tape strips) was 21.50%, 58.42% and 40.39%, respectively (Table 5, Figures 6-7); 98.81% of the applied radioactivity was recovered at the end of the 24-hour exposure phase.

These data show that following application of a 0.4% butylparaben emulsion to viable, full-thickness human skin, that of the total radioactivity detected in the receptor fluid (21.01%), almost all was accounted for as the principle metabolite 4-hydroxybenzoic acid.

CONCLUSIONS

Following application of a 0.4% butylparaben emulsion to viable, full-thickness human skin, total penetration at 24 hours post-exposure was 21.01%. The principle metabolite, 4-hydroxybenzoic acid, was detected in the receptor fluid over the course of the exposure phase with barely detectable levels of unmetabolized butylparaben in receptor fluid from only one of the six skin replicates.

Overall these data suggest that the dermal bioavailability of butylparaben from an oil-in-water emulsion was low and that first pass metabolism resulted in complete hydrolysis to the primary acid metabolite.

RECORDS AND SAMPLE STORAGE

All data and records for analytical characterizations conducted by the sponsor will be retained by the sponsor. Raw data and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware, and will be returned to the sponsor within 6 months after the final report issues.

REFERENCES

1. Scott, R.C., Batten, P.L., Clowes, H.M., Jones, B.K., and Ramsey, J.D. (1992). Further Validation of an In Vitro Method to Reduce the Need for In Vivo Studies for Measuring the Absorption of Chemicals through Rat Skin. Fundamental and Applied Toxicology 19, 484-492.

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3. Scott, R.C., Walker, M., and Dugard, P.H. (1986). A comparison of the in vitro permeability properties of human and some laboratory animal skins. International Journal of Cosmetic Science 8, 189-194.

4. The Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers (2003). Basic Criteria for the In Vitro Assessment of Dermal Absorption of Cosmetic Ingredients (SCCNFP/0750/03).

5. Fasano, W.J., Manning, L.A., and Green, J.W. (2002). Rapid Integrity Assessment of Rat and Human Epidermal Membranes for In Vitro Dermal Regulatory Testing: Correlation of Electrical Resistance with Tritiated Permeability. Toxicology In Vitro 16, 731-740.

6. Fasano, W.J., Hinderliter, P.M. (2004). The Tinsley LCR Databridge Model 6401 and electrical impedance measurements to evaluate skin integrity in vitro. Toxicology In Vitro 18, 725-729.

TABLES

TABLES

EXPLANATORY NOTES

ABBREVIATIONS:

absorbed	receptor fluid + receptor wash
absorbable	absorbed + skin
equiv	equivalent
SD	standard deviation
unabsorbed	skin wash + donor chamber + tape strips

Table 1: Application amounts and rates

	Mean	SD
Activity applied (μCi)	0.85	0.05
Total Butylparaben (μg)	20.41	1.16
Butylparaben per area (μg/cm^2)	31.89	1.81

Table 2: Cumulative amount absorbed per area

Time (hours)	Radioactivity (µg equiv/cm^2)		Butylparaben (µg/cm^2)		4-hyroxybenzoic acid (µg/cm^2)	
	Mean	SD	Mean[a]	SD	Mean	SD
1	NA	NA	NA	NA	0.05	0.01
2	0.01	NA	NA	NA	0.11	0.03
3	0.03	0.03	NA	NA	0.16	0.05
4	0.09	0.08	NA	NA	0.28	0.10
5	0.21	0.15	NA	NA	0.42	0.16
6	0.38	0.25	NA	NA	0.60	0.22
8	0.86	0.47	<0.00	NA	1.09	0.44
10	1.47	0.74	<0.02	NA	1.69	0.64
12	2.15	0.97	<0.06	NA	2.71	1.07
14	2.90	1.23	<0.09	NA	3.70	1.27
16	3.66	1.48	<0.12	NA	4.54	1.49
18	4.44	1.72	<0.14	NA	5.49	1.69
20	5.21	1.96	<0.17	NA	6.36	1.90
22	5.98	2.19	<0.19	NA	7.01	2.03
24[b]	6.74	2.39	<0.22	NA	7.69	2.24

[a]Butylparaben detected in only 1 of 6 cells
[b]Cumulative amount absorbed at 24 hours does not include receptor wash.

Table 3: Concentration-time course

Time (hours)	Radioactivity (µg equiv/mL)		Butylparaben (µg/mL)		4-hyroxybenzoic acid (µg/mL)	
	Mean	SD	Mean[a]	SD	Mean	SD
1	NA	NA	NA	NA	0.01	0.00
2	0.00	NA	NA	NA	0.02	0.00
3	0.01	0.01	NA	NA	0.02	0.01
4	0.02	0.01	NA	NA	0.03	0.01
5	0.03	0.02	NA	NA	0.03	0.02
6	0.04	0.02	NA	NA	0.04	0.02
8	0.06	0.03	<0.000	NA	0.06	0.03
10	0.07	0.03	<0.002	NA	0.07	0.03
12	0.08	0.03	<0.006	NA	0.13	0.06
14	0.09	0.03	<0.003	NA	0.12	0.03
16	0.09	0.03	<0.004	NA	0.10	0.03
18	0.10	0.03	<0.002	NA	0.12	0.04
20	0.10	0.03	<0.004	NA	0.11	0.03
22	0.09	0.03	<0.002	NA	0.08	0.02
24[b]	0.09	0.03	<0.004	NA	0.08	0.03

[a]Butylparaben detected in only 1 of 6 cells
[b]Cumulative amount absorbed at 24 hours does not include receptor wash.

Table 4: Cumulative percent absorbed

Time	Radioactivity		Butylparaben		4-hyroxybenzoic acid	
(hours)	Mean	SD	Mean[a]	SD	Mean	SD
1	NA	NA	NA	NA	0.14	0.05
2	0.04	NA	NA	NA	0.34	0.11
3	0.09	0.09	NA	NA	0.51	0.15
4	0.28	0.25	NA	NA	0.87	0.30
5	0.65	0.47	NA	NA	1.31	0.46
6	1.19	0.76	NA	NA	1.87	0.65
8	2.67	1.43	<0.01	NA	3.40	1.33
10	4.56	2.22	<0.06	NA	5.26	1.89
12	6.68	2.91	<0.20	NA	8.46	3.18
14	9.02	3.66	<0.27	NA	11.55	3.71
16	11.39	4.36	<0.36	NA	14.18	4.34
18	13.81	5.06	<0.42	NA	17.13	4.89
20	16.23	5.73	<0.52	NA	19.85	5.46
22	18.63	6.38	<0.57	NA	21.87	5.81
24[b]	21.01	6.95	<0.67	NA	24.00	6.38

[a]Butylparaben detected in only 1 of 6 cells
[b]Cumulative amount absorbed at 24 hours does not include receptor wash.

Table 5: Material balance

		Mean	SD
Absorbed dose			
	Receptor fluid	21.01	6.95
	Receptor wash	0.49	0.16
	Total absorbed	21.50	7.06
Absorbable dose			
	Receptor fluid	21.01	6.95
	Receptor wash	0.49	0.16
	Skin	36.92	4.97
	Total absorbable	58.42	10.39
Unabsorbed dose			
	Skin wash	37.85	8.12
	Donor chamber	0.82	0.46
	Tape strips	1.72	0.40
	Total unabsorbed	40.39	8.10
Total recovered		98.81	3.45

FIGURES

FIGURES

EXPLANATORY NOTES

ABBREVIATIONS:

CPM	count per minute
DPM	disintegrations per minute
h	hours

Figure 1: ^{14}C-butylparaben, Flo-one radiochromatogram, neat

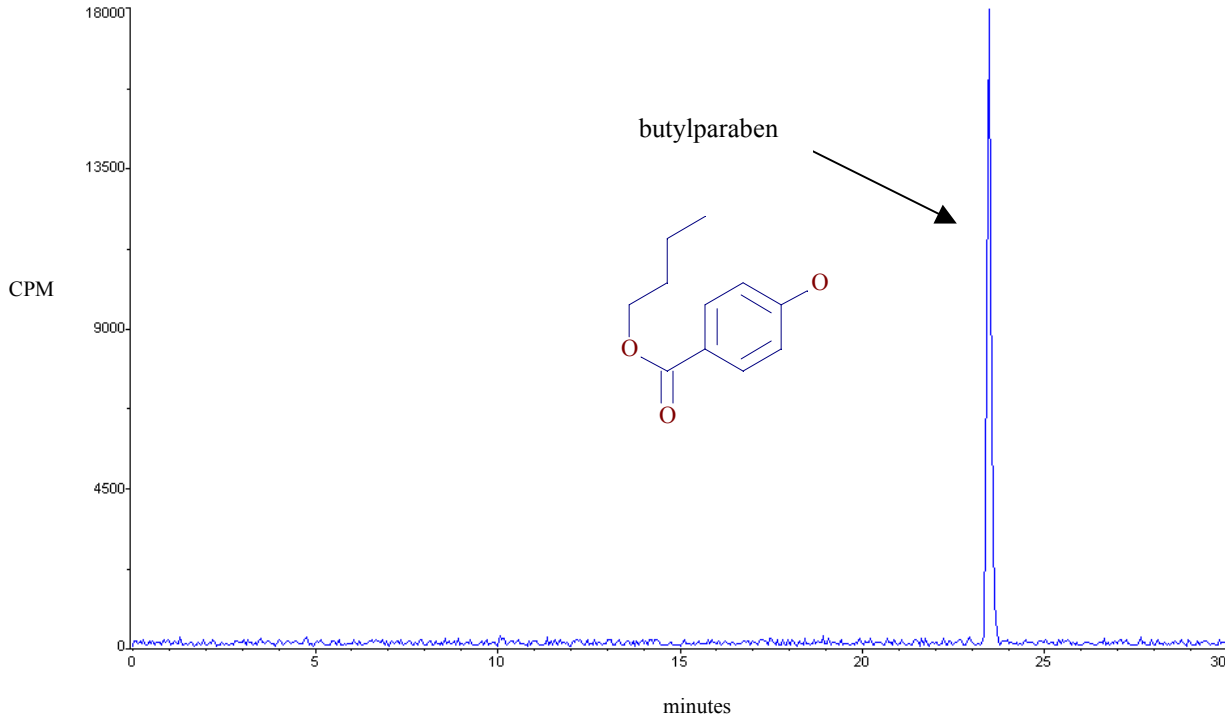


Figure 2: Representative radiochromatogram ^{14}C-butylparaben in 0.4% emulsion

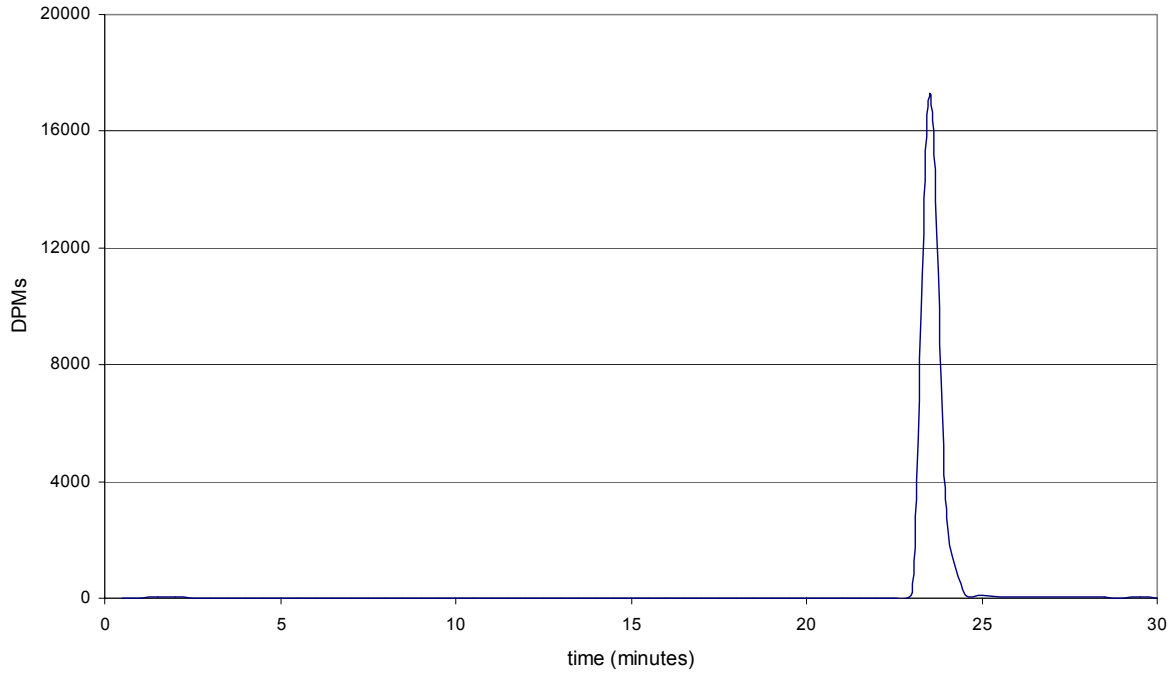


Figure 3: Cumulative amount absorbed per area, total radioactivity, butylparaben, 4-hydroxybenzoic acid

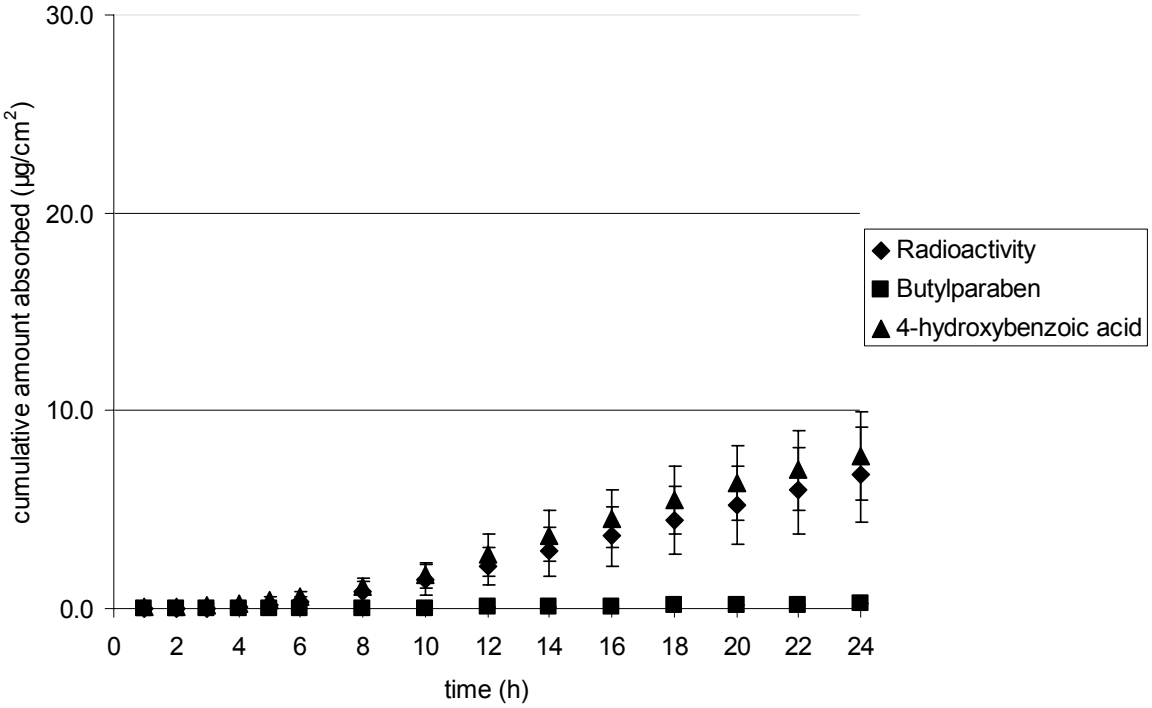


Figure 4: Concentration-time course, total radioactivity, butylparaben, 4-hydroxybenzoic acid

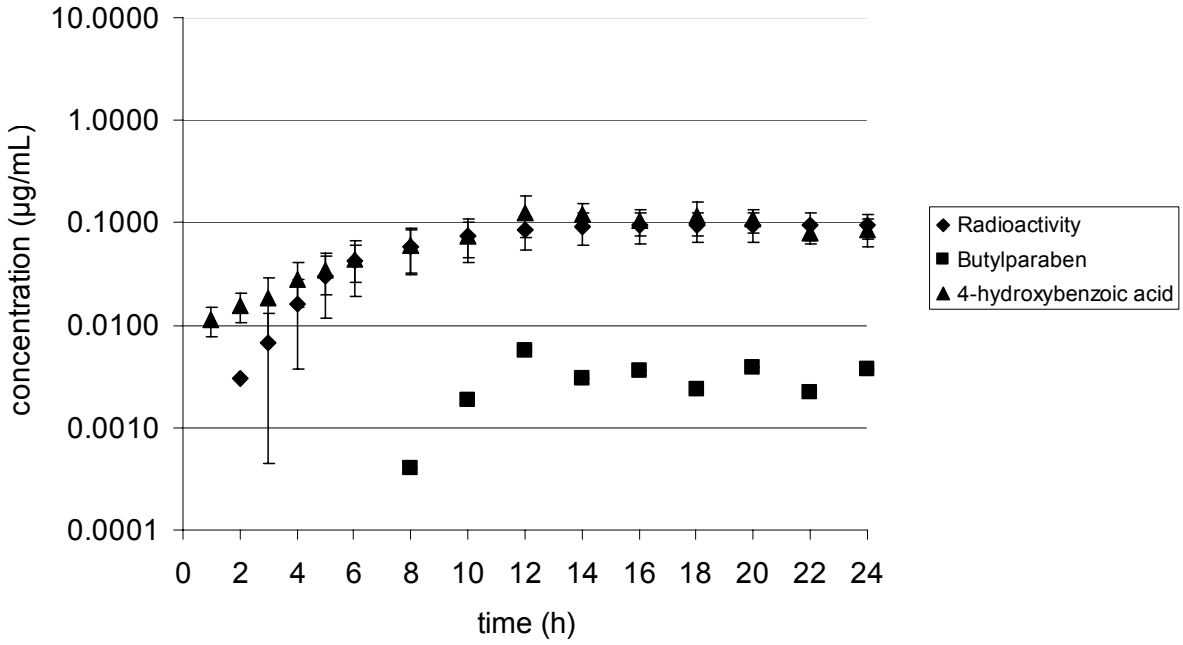


Figure 5: Cumulative percent absorbed, total radioactivity, butylparaben, 4-hydroxybenzoic acid

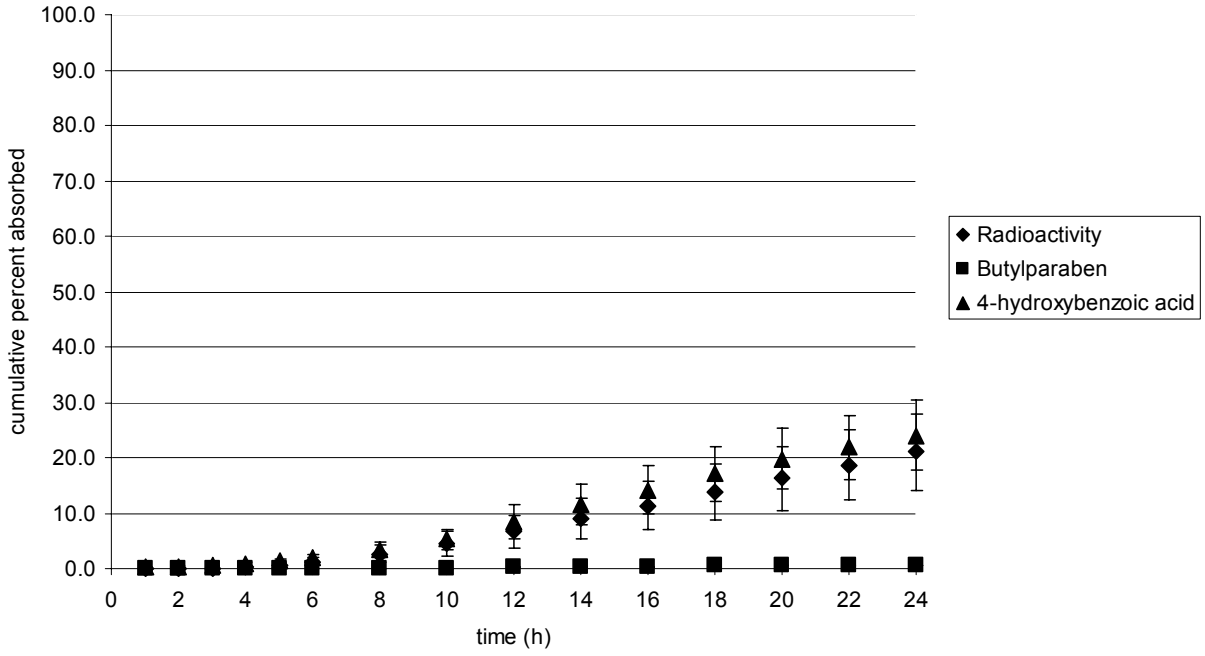


Figure 6: Total radioactivity, material balance

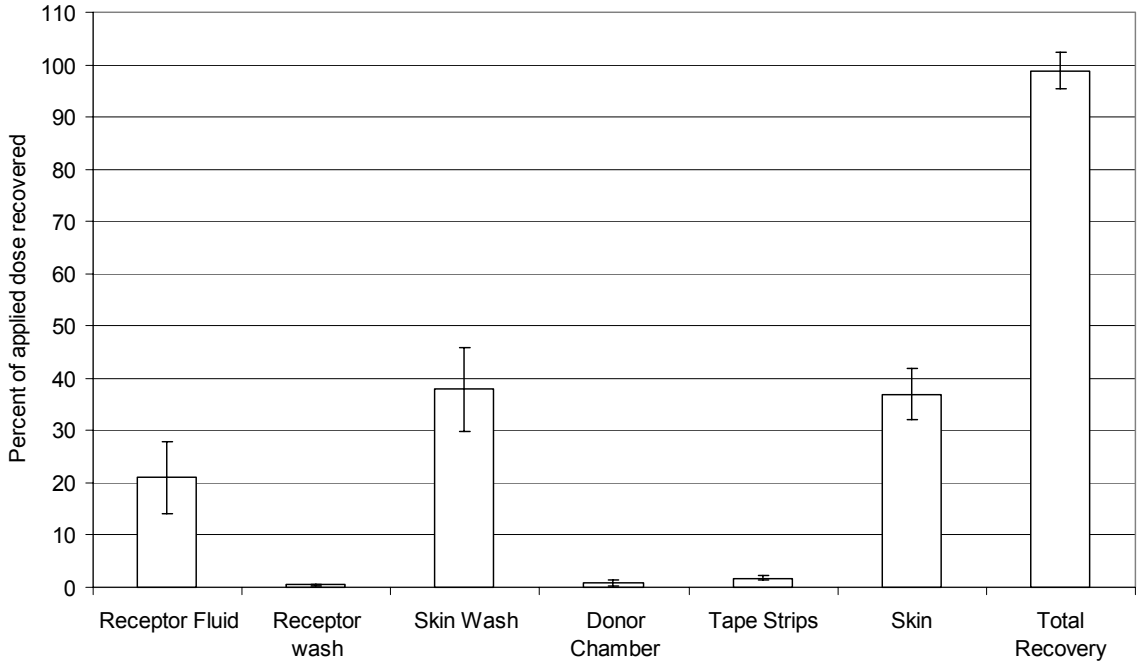
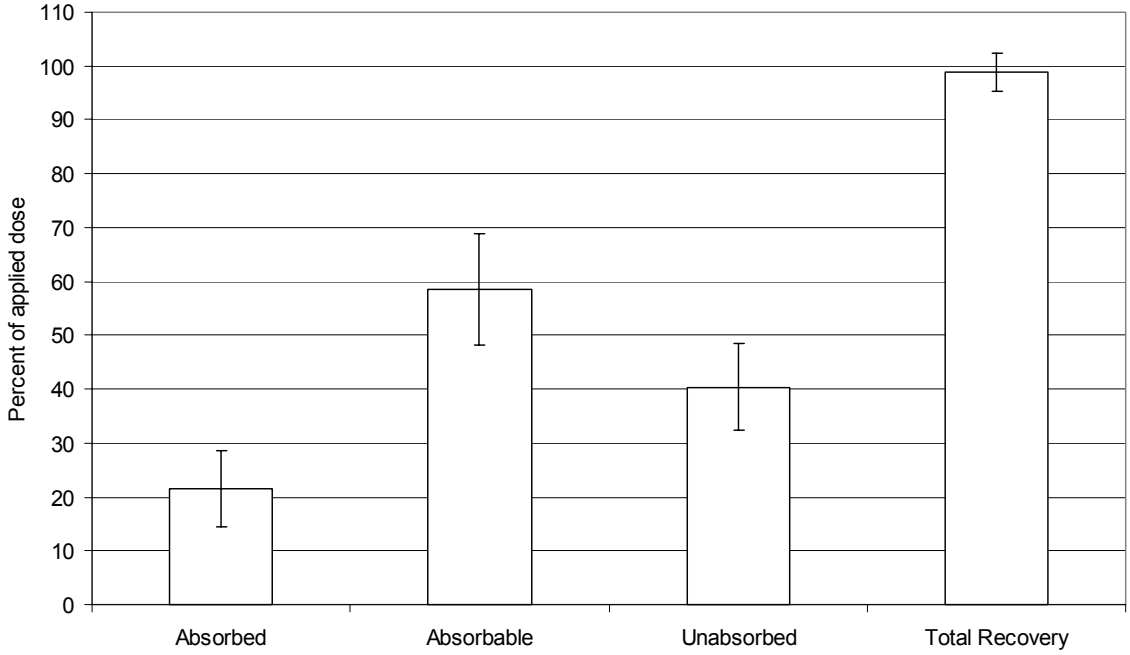


Figure 7: Absorbed, absorbable, unabsorbed, total recovered



APPENDICES

APPENDICES

EXPLANATORY NOTES

ABBREVIATIONS:

absorbed	receptor fluid + receptor wash
absorbable	absorbed + skin
BP	butylparaben
equiv	equivalent
h	hour(s)
NA	not applicable
SD	standard deviation
unabsorbed	skin wash + donor chamber + tape strips

APPENDIX A

Certificates of Analysis

PROTAMEEN CHEMICALS INC.

375 Minnisink Road
Totowa, N.J. 07511
Office: 973-256-4374
Fax: 973-256-6764

Certificate of Analysis

Product Number: 330
Product Name: BUTYL PARABEN
Lot Number: B3140

Customer Name:
Customer PO#:
Customer Product Code:
Customer Prod Name:

Test Name:	Range:	Result:
Appearance @ 25dgr	White crystalline powder	White crystalline powder
Assay (%)	99.0 - 100.5	99.5
Identification - IR	Complies with standard	Pass
Melting Range Celcius	68 - 72	70
Acidity	Complies wiith standard	Conforms
Loss on Drying %	0.5 Maximum	0.02
Residue on Ignition	0.05 Maximum	0.05
Organic Volatile Impurities	Complies with standard	Will Comply

Expiration Date: 2/10/2006
Manufacture Date: 2/11/2003
Issue Date: 10/14/2003
Signature: JOHN J. BRODZINSK
TECHNICAL DIRECTO

Remarks: MANUFACTURED BY UENO FINE CHEMICALS

Safety data sheet **Date of issue 15 January 2004**

Amersham Biosciences UK Limited Amersham Place Little Chalfont
Buckinghamshire HP7 9NA UK Telephone: +44 (0)870 606 1921

[redacted]

Product name : 4-Hydroxybenzoic acid butyl ester CAS No. 94-26-8 EC No. 202-318-7

S: 22-24/25 Do not breathe dust. Avoid contact with skin and eyes.

Composition : 4-Hydroxybenzoic acid butyl ester is supplied as a radioactive solid.

Hazards identification : 4-Hydroxybenzoic acid butyl ester is not classified as hazardous.

First aid measures : In case of contact, immediately flush eyes and skin with water. If inhaled, remove to fresh air. If swallowed, wash out mouth with water. In severe cases, or in case of contact with eyes, seek medical attention.

Fire fighting measures : For small fires only. Wear protective clothing. Use water spray, carbon dioxide, dry chemical powder or appropriate foam.

Accidental release : Avoid exposure to dust. Wear protective clothing including laboratory overalls, safety glasses and gloves. Treat as for spills of radioactive material (see Handling Instructions for Radioactive materials).

Handling and storage : Follow handling instructions for Radioactive materials. Wear protective clothing including laboratory overalls, safety glasses and gloves. Use in a chemical fume hood. Do not breathe dust. Avoid contact with skin and eyes. Avoid prolonged or repeated exposure. Keep container tightly closed. Wash thoroughly after handling.

Personal protection: See above instructions for handling and storage.

Physical and chemical properties : Form and appearance : solid
Melting point : 68°C
Boiling point : 156-157°C (3.5 mmHg)

Stability and reactivity : 4-Hydroxybenzoic acid butyl ester is stable.
Avoid strong oxidising agents.

Toxicological information : LD_{50}: 13200mg/kg oral, mouse.
May cause skin and eye irritation. May be harmful if absorbed through the skin, inhaled or swallowed. Material may be irritating to mucous membranes and upper respiratory tract.

Ecological Information : Not available.

Disposal considerations : Dispose of waste material as for radioactive waste.
(See: Instructions relating to the Handling and Disposal of Radioactive Materials.)

Transport information : As applicable to radioactive materials.

Regulatory information : The information contained in this Safety Data Sheet is based on published sources of information and is believed to be correct. It should be used a guide only. It is the responsibility of the user of this product to carry out an assessment of workplace risks, as may be required under national legislation.

CAUTION - RADIOACTIVE MATERIAL

Product Specification

http://www.customlabeling.com
Amersham Biosciences UK Limited
Amersham Place Little Chalfont Buckinghamshire HP7 9NA UK
Telephone +44 (0)870 606 1921

Certificate number LRQ 0925281

Before using this product, please read the instructions overleaf for safe handling, storage and disposal

4-Hydroxy[ring-U-^{14}C]benzoic acid butyl ester

Code CFQ13766 Batch 1 [redacted]
Pack size 185 MBq, 5mCi

Technical data

Specific activity			
determined by mass spectrometry	:	518 MBq/mmol,	14 mCi/mmol
determined by gravimetric analysis	:	2.78 MBq/mg	75 µCi/mg
Molecular weight	:	194.7 (at this specific activity)	
Radiochemical purity			
by high performance liquid chromatography	:	99.2%	

Column	:	Inertsil ODS 3V 5µm (250 x 4.6mm)					
Solvent A	:	0.1% trifluoroacetic acid in water					
Solvent B	:	0.1% trifluoroacetic acid in acetonitrile					
Gradient	:	Time (mins)	0	15	30	31	35
		% B	0	100	100	0	0
Flow rate	:	1 ml/min					
UV detection	:	254 nm					

Analysed on 29th January 2004

Chemical identity

The material co-chromatographs with commercially available material in the above chromatographic system.

The mass spectrum is consistent with the proposed structure and a non-labelled reference.

The ^{1}H-nmr spectrum is consistent with the proposed structure and a non-labelled reference.

Amersham Biosciences

Cosmetech
LABORATORIES, INC.
39 PLYMOUTH STREET • SUITE 4 • FAIRFIELD, NEW JERSEY 07004-1681
TELEPHONE (973) 882-5151 • FAX (973) 882-1222
E-MAIL Ken@Cosmetech.com E-MAIL Irwin@Cosmetech.com

KENNETH KLEIN
PRESIDENT

IRWIN PALEFSKY
SR. VICE PRESIDENT

March 1, 2004

Mr. Bill Fasano
Stine Haskell Research Center
1090 Elkton Road
Building Haskell 1 Room 620
Newark, DE 19714

Dear Mr. Fasano:

Enclosed are the phases necessary to make the Oil in Water emulsion formulation that you will use for the incorporation of parabens.

Please let us know if you have any questions.

Best regards,

COSMETECH LABORATORIES, INC.

Irwin Palefsky
Sr. Vice President

IP/fh
Encl.

COSMETECH LABORATORIES, INC.

PRODUCT: PREMIX EMULSION FOR PARABEN STUDY
PURPOSE: TO PREPARE AN OIL-IN-WATER EMULSION FOR PARABEN INCORPORATION
PREPARED FOR: CTFA LAB #CLI: 1662701 DATE: 03/01/04
PREPARED BY: NW SUPERSEDES: DATE:
APPROVED BY: K.KLEIN

NO.	PHASE	INGREDIENT (TRADE NAME)	INCI DESIGNATION	SUPPLIER	% BY WEIGHT	BATCH SIZE
						1500.00
1	A	WATER	WATER (AQUA)		76.00	1140.00
2	A	GLYCERIN 96%	GLYCERIN	COGNIS	3.00	45.00
3	A	CARBOPOL 980 (2% SOLN)	CARBOMER	NOVEON	7.50	112.50
4	B	STEARIC ACID XXX	STEARIC ACID	COGNIS	4.00	60.00
5	B	CETYL ALCOHOL	CETYL ALCOHOL	COGNIS	0.25	3.75
6	B	STEARYL ALCOHOL	STEARYL ALCOHOL	COGNIS	0.25	3.75
7	B	DRAKEOL #7	#N/A	#N/A	5.00	75.00
8	B	CERASYNT Q	GLYCERYL STEARATE SE	ISP	2.00	30.00
9	C	TRIETHANOLAMINE 99%	TRIETHANOLAMINE	UNION CARBIDE	1.20	18.00
					—	—
		TOTAL			99.20	1488.00

MANUFACTURING INSTRUCTIONS:
FOR FINAL FORMULA PREPARATION HEAT PHASE A TO 75C. ADD PARABENS TO PHASE A.HEAT PHASE B TO 75C.
ADD PHASE B TO PHASE A. ADD PHASE C TO PHASE A/B. COOL WITH SLOW MIXING UNTIL
TEMPERATURE REACHES 35C. PACKAGE AT 35C.

LAB CONTROL:
pH: 6.50-7.25
OTHER INFORMATION:

VISCOSITY (CPS): 12,500 CPS- 25,000 CPS

H# 22703-289

SIGMA-ALDRICH

3050 Spruce Street
Saint Louis, Missouri 63105 USA
Telephone (800) 521-8956 • (314) 771-5765
Fax (800) 325-5052 • (314) 77-5757
Visit Us At www.sigma-aldrich.com

Certificate of Analysis

DUPONT
JULIA WANG, H1/1313
DUPONT - STINE-HASKELL/B1
ELKTON RD
NEWARK DE 19711

PO NBR: PC294063

PRODUCT NUMBER: W220302-SPEC LOT NUMBER: 12116CU

PRODUCT NAME: BUTYL P-HYDROXYBENZOATE, 99+%

FORMULA: C11H14O3 FORMULA WEIGHT: 194.23

APPEARANCE	WHITE CRYSTALLINE POWDER
MELTING POINT	69.1-70.3 DEGREES CELSIUS
INFRARED SPECTRUM	CONFORMS TO STRUCTURE AND STANDARD AS ILLUSTRATED ON PAGE 620C OF EDITION I, VOLUME 1 OF "THE ALDRICH LIBRARY OF FT-IR SPECTRA".
GAS LIQUID CHROMATOGRAPHY	99.9 %
SOLUBILITY	5% IN 95% ETHANOL; CLEAR, COLORLESS SOLUTION
QUALITY CONTROL ACCEPTANCE DATE	MARCH 1999

ALDRICH CHEMICAL COMPANY
RONNIE MARTIN
DECEMBER 11, 2003

*We are Committed to the success of our Customers, Employees and Shareholders through leadership in **Life Science, High Technology** and Service.*

H# 22703-290

SIGMA-ALDRICH

3050 Spruce Street
Saint Louis, Missouri 63105 USA
Telephone (800) 521-8956 • (314) 771-5765
Fax (800) 325-5052 • (314) 77-5757
Visit Us At www.sigma-aldrich.com

Certificate of Analysis

PO NBR:

PRODUCT NUMBER: W39860-8 LOT NUMBER: 26026BB

PRODUCT NAME: P-HYDROXYBENZOIC ACID, 99+%

FORMULA: C7H6O3 FORMULA WEIGHT: 138.12

APPEARANCE	WHITE POWDER
MELTING POINT	215.5-217.3 DEGREES CELSIUS
INFRARED SPECTRUM	CONFORMS TO STRUCTURE.
TITRATION	100.8 % (WITH NAOH)
GAS LIQUID CHROMATOGRAPHY	99.9 %
SOLUBILITY	50MG/ML, MEOH, VERY SLIGHTLY HAZY,COLORLESS SOLUTION
QUALITY CONTROL ACCEPTANCE DATE	MARCH, 2003

ALDRICH CHEMICAL COMPANY
RONNIE MARTIN
DECEMBER 22, 2003

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APPENDIX B

Butylparaben – Human

Application amounts and rates

Skin Replicate	Activity applied (μCi)	Total BP (μg)	BP per area (μg/cm^2)
1	0.80	19.3	30.2
2	0.90	21.7	33.9
3	0.86	20.7	32.4
4	0.89	21.3	33.3
5	0.86	20.7	32.3
6	0.78	18.7	29.3
7	0.96	23.2	36.2
8	0.95	22.9	35.8
9	0.96	23.1	36.2
10	0.92	22.2	34.7
11	0.94	22.7	35.4
12	0.94	22.5	35.1
13	0.92	22.1	34.5
Mean	0.85	20.41	31.89
SD	0.05	1.16	1.81

Cumulative amount of radioactivity absorbed per area (µg equiv/cm^2)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1	NA	NA	0.01	0.06	0.15	0.29	0.69	1.15	1.71	2.33	2.95	3.57	4.16	4.78	5.40
2	NA	NA	0.01	0.06	0.15	0.29	0.67	1.18	1.77	2.42	3.09	3.80	4.50	5.21	5.91
3	NA	0.01	0.08	0.25	0.51	0.86	1.74	2.81	3.87	5.04	6.17	7.31	8.42	9.54	10.63
4	NA	NA	0.02	0.09	0.23	0.43	1.02	1.79	2.68	3.67	4.68	5.71	6.77	7.77	8.70
5	NA	NA	0.02	0.06	0.15	0.28	0.65	1.13	1.70	2.29	2.90	3.54	4.18	4.81	5.45
6	NA	NA	NA	0.02	0.07	0.15	0.39	0.73	1.17	1.67	2.18	2.71	3.24	3.80	4.39
MEAN	NA	0.01	0.03	0.09	0.21	0.38	0.86	1.47	2.15	2.90	3.66	4.44	5.21	5.98	6.74
SD	NA	NA	0.03	0.08	0.15	0.25	0.47	0.74	0.97	1.23	1.48	1.72	1.96	2.19	2.39

Concentration-time course of total radioactivity (µg equiv/mL)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1	NA	NA	0.00	0.01	0.02	0.03	0.05	0.06	0.07	0.08	0.08	0.08	0.08	0.08	0.08
2	NA	NA	0.00	0.01	0.02	0.03	0.05	0.06	0.07	0.08	0.08	0.09	0.09	0.09	0.09
3	NA	0.00	0.02	0.04	0.06	0.09	0.11	0.13	0.13	0.14	0.14	0.14	0.14	0.14	0.13
4	NA	NA	0.01	0.02	0.03	0.05	0.07	0.10	0.11	0.12	0.12	0.13	0.13	0.12	0.12
5	NA	NA	0.00	0.01	0.02	0.03	0.05	0.06	0.07	0.07	0.08	0.08	0.08	0.08	0.08
6	NA	NA	NA	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.06	0.06	0.07	0.07	0.07
MEAN	NA	0.00	0.01	0.02	0.03	0.04	0.06	0.07	0.08	0.09	0.09	0.10	0.10	0.09	0.09
SD	NA	NA	0.01	0.01	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03

Cumulative percent of radioactivity absorbed

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1	NA	NA	0.05	0.20	0.51	0.98	2.27	3.80	5.57	7.62	9.66	11.71	13.78	15.83	17.87
2	NA	NA	0.04	0.17	0.43	0.85	1.98	3.48	5.23	7.12	9.11	11.20	13.25	15.34	17.40
3	NA	0.04	0.26	0.76	1.56	2.65	5.36	8.66	11.95	15.55	19.04	22.56	25.98	29.41	32.78
4	NA	NA	0.06	0.28	0.70	1.31	3.06	5.39	8.07	11.04	14.06	17.18	20.35	23.35	26.16
5	NA	NA	0.05	0.19	0.48	0.85	2.01	3.51	5.25	7.09	8.98	10.96	12.94	14.88	16.86
6	NA	NA	NA	0.07	0.24	0.50	1.33	2.51	4.00	5.72	7.46	9.26	11.09	12.97	15.00
MEAN	NA	0.04	0.09	0.28	0.65	1.19	2.67	4.56	6.68	9.02	11.39	13.81	16.23	18.63	21.01
SD	NA	NA	0.09	0.25	0.47	0.76	1.43	2.22	2.91	3.66	4.36	5.06	5.73	6.38	6.95

Material Balance

Cell ID (replicate)	Receptor Fluid	Receptor wash	Skin Wash	Donor Chamber	Tape Strips	Skin	Total Recovery
1	17.87	0.32	48.55	0.54	1.79	31.06	100.14
2	17.40	0.32	42.16	0.26	1.37	34.74	96.25
3	32.78	0.63	29.71	0.90	2.24	37.03	103.29
4	26.16	0.71	26.94	0.71	1.15	46.05	101.72
5	16.86	0.41	41.07	0.91	1.87	35.99	97.10
6	15.00	0.54	38.65	1.62	1.91	36.63	94.35
MEAN	21.01	0.49	37.85	0.82	1.72	36.92	98.81
SD	6.95	0.16	8.12	0.46	0.40	4.97	3.45

Cell ID (replicate)	Absorbed	Absorbable	Unabsorbed
1	18.20	49.36	50.88
2	17.72	52.46	43.79
3	33.41	70.44	32.85
4	26.87	72.92	28.80
5	17.28	53.27	43.84
6	15.55	52.18	42.17
MEAN	21.50	58.42	40.39
SD	7.06	10.39	8.10

Cumulative amount of butylparaben absorbed per area (µg/cm^2)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	NA	NA	NA	NA	NA	NA	0.00	0.02	0.06	0.09	0.12	0.14	0.17	0.19	0.22
4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
MEAN	NA	NA	NA	NA	NA	NA	<0.00	<0.02	<0.06	<0.09	<0.12	<0.14	<0.17	<0.19	<0.22
SD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Concentration-time course of butylparaben (µg equiv/mL)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	NA	NA	NA	NA	NA	NA	0.00	0.002	0.006	0.003	0.004	0.002	0.004	0.002	0.004
4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
MEAN	NA	NA	NA	NA	NA	NA	<0.00	<0.002	<0.006	<0.003	<0.004	<0.002	<0.004	<0.002	<0.004
SD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Cumulative percent of butylparaben absorbed

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	NA	NA	NA	NA	NA	NA	0.01	0.06	0.20	0.27	0.36	0.42	0.52	0.57	0.67
4	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
MEAN	NA	NA	NA	NA	NA	NA	<0.01	<0.06	<0.20	<0.27	<0.36	<0.42	<0.52	<0.57	<0.67
SD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Cumulative amount of 4-hydroxybenzoic acid absorbed per area (µg/cm^2)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1	NA	NA	0.11	0.20	0.30	0.47	0.97	1.43	2.33	3.11	3.82	4.58	5.29	5.93	6.46
2	0.02	0.05	0.13	0.26	0.38	0.51	0.93	1.67	2.52	3.51	4.19	4.91	5.83	6.47	7.18
3	0.05	0.12	0.26	0.48	0.72	1.03	1.96	2.89	4.79	6.10	7.30	8.41	9.63	10.45	11.47
4	0.04	0.12	0.16	0.26	0.44	0.61	1.06	1.71	2.71	3.91	5.00	6.57	7.58	8.36	9.17
5	0.05	0.12	0.17	0.23	0.36	0.54	0.88	1.42	2.05	3.11	3.90	4.65	5.34	5.87	6.47
6	0.06	0.13	0.15	0.25	0.32	0.43	0.73	1.00	1.85	2.48	3.06	3.82	4.50	4.96	5.40
MEAN	0.05	0.11	0.16	0.28	0.42	0.60	1.09	1.69	2.71	3.70	4.54	5.49	6.36	7.01	7.69
SD	0.01	0.03	0.05	0.10	0.16	0.22	0.44	0.64	1.07	1.27	1.49	1.69	1.90	2.03	2.24

Concentration-time course of 4-hydroxybenzoic acid (µg equiv/mL)

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1	NA	NA	0.03	0.02	0.02	0.04	0.06	0.06	0.11	0.10	0.09	0.09	0.09	0.08	0.07
2	0.01	0.01	0.02	0.03	0.03	0.03	0.05	0.09	0.10	0.12	0.08	0.09	0.11	0.08	0.09
3	0.01	0.02	0.03	0.05	0.06	0.08	0.12	0.11	0.23	0.16	0.15	0.14	0.15	0.10	0.13
4	0.01	0.02	0.01	0.03	0.04	0.04	0.05	0.08	0.12	0.15	0.13	0.19	0.12	0.10	0.10
5	0.01	0.02	0.01	0.02	0.03	0.04	0.04	0.07	0.08	0.13	0.10	0.09	0.09	0.07	0.07
6	0.02	0.02	0.01	0.02	0.02	0.03	0.04	0.03	0.10	0.08	0.07	0.09	0.08	0.06	0.05
MEAN	0.01	0.02	0.02	0.03	0.03	0.04	0.06	0.07	0.13	0.12	0.10	0.12	0.11	0.08	0.08
SD	0.00	0.00	0.01	0.01	0.02	0.02	0.03	0.03	0.06	0.03	0.03	0.04	0.03	0.02	0.03

Cumulative percent of 4-hydroxybenzoic acid absorbed

Cell ID	Time after dosing (h)														
(replicate)	1	2	3	4	5	6	8	10	12	14	16	18	20	22	24
1	NA	NA	0.37	0.65	0.98	1.56	3.22	4.75	7.71	10.30	12.64	15.18	17.53	19.65	21.39
2	0.07	0.16	0.40	0.78	1.13	1.49	2.73	4.93	7.43	10.33	12.34	14.48	17.17	19.07	21.16
3	0.14	0.37	0.80	1.47	2.22	3.16	6.05	8.90	14.78	18.82	22.51	25.94	29.70	32.24	35.38
4	0.12	0.36	0.48	0.79	1.33	1.85	3.19	5.14	8.16	11.74	15.03	19.73	22.78	25.13	27.58
5	0.16	0.37	0.52	0.72	1.12	1.67	2.72	4.40	6.36	9.62	12.08	14.40	16.55	18.19	20.03
6	0.22	0.44	0.51	0.84	1.10	1.46	2.51	3.42	6.32	8.48	10.46	13.04	15.39	16.97	18.47
MEAN	0.14	0.34	0.51	0.87	1.31	1.87	3.40	5.26	8.46	11.55	14.18	17.13	19.85	21.87	24.00
SD	0.05	0.11	0.15	0.30	0.46	0.65	1.33	1.89	3.18	3.71	4.34	4.89	5.46	5.81	6.38

CHARLES RIVER
LABORATORIES
Discovery and Development Services
Argus Division

REPORT AMENDMENT 1
15 APRIL 2005

PROTOCOL 1203-006

ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

FINAL REPORT

1. Section 1.1. Study Design (page 5 of the report), first paragraph, third sentence was changed to read:

   "All rats were given test diets when weaned on day 21 postpartum. Exposure continued until the day of sacrifice."

   rather than

   "All rats were 22 days of age when first exposed to test diets, with exposure continuing until the day of sacrifice."

2. Section 2.8.4. Method and Frequency of Administration (page 19 of the report), the first paragraph was changed to read:

   "A carrier control and three test diet concentrations were given to the rats. Rats were given continual access to either the carrier control diet (Group I) or the test article in the carrier control diet (Groups II to IV) for at least 56 days beginning on day 21 postpartum. All exposures were continued to the day of sacrifice."

   rather than

   "A carrier control and three test diet concentrations were given to the rats. Rats (in Groups II through IV) were given continual access to the test article in the diet for at least 56 days beginning on day 21 postpartum. All exposures were continued to the day of sacrifice."

905 Sheehy Drive, Bldg. A, Horsham, PA 19044 • 215.443.8710 • FAX 215.443.8587

These changes were made to clarify the exposure period for the male rats and did not affect the results of the study.

4/15/05

Alan M. Hoberman, Ph.D., DABT Date
Director of Research
and Study Director

15 Apr 05

Cynthia M. Kelsch, LAT Date
Quality Assurance Supervisor

TITLE: ORAL (DIET) REPRODUCTION TOXICITY STUDY OF BUTYLPARABEN IN MALE RATS

CR-DDS ARGUS DIVISION PROTOCOL NUMBER: 1203-006

1. SUMMARY AND CONCLUSION

The purpose of this study was to test for toxic effects/disturbances resulting from oral (diet) exposure to butylparaben of Crl:(WI) BR male rats on spermatogenesis.

1.1. Study Design[a]

Sixty-four male rats were assigned to four exposure groups, 16 male rats per group. Prepared diets containing the test article, butylparaben, at constant concentrations of 0, 100, 1000 and 10000 ppm were available *ad libitum* to the rats for a minimum of 56 days. All rats were given test diets when weaned on day 21 postpartum. Exposure continued until the day of sacrifice. Viabilities, clinical observations, body weights and feed consumption values were recorded. Beginning at the start of week 3 of the exposure period, blood samples were collected every other week from each male rat assigned to study and analyzed at the Testing Facility for LH (luteinizing hormone), FSH (follicle-stimulating hormone) and testosterone.

On the day of sacrifice, all surviving F1 generation male rats were sacrificed, and blood samples were collected for possible future analysis for butylparaben and para hydroxy benzoic acid levels. A gross necropsy of the thoracic, abdominal and pelvic viscera was performed. Reproductive organs from all rats, as well as the liver, adrenal glands, thyroid and pituitary gland (six per group), were weighed and retained for possible histological evaluation. Sperm evaluations were conducted to determine sperm concentration, motility and morphology. The left testis from each rat was collected for evaluation of Daily Sperm Production (DSP) determinations (i.e. testicular spermatid concentration). The liver, adrenal, thyroid and pituitary glands from ten male rats per exposure group were quick-frozen in liquid nitrogen and retained for possible hormone measurements. Histological examination was performed on the reproductive organs from all rats assigned to the control and high test article concentration groups. Additionally, the liver, adrenals, thyroid and pituitary glands from six rats in the control and high test article concentration groups were evaluated. A detailed qualitative examination of the testes was conducted, taking into account the tubular stages of the spermatogenic cycle. A gross necropsy was conducted on the rats that were sacrificed due to moribund condition, and protocol-specified tissues were retained for histological examination.

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a. Detailed descriptions of all procedures used in the conduct of this study are provided in the appropriate sections of this report and in APPENDIX C (PROTOCOL).

A carrier control and three test diet concentrations were given to the rats. Rats were given continual access to either the carrier control diet (Group I) or the test article in the carrier control diet (Groups II to IV) for at least 56 days beginning on day 21 postpartum. All exposures were continued to the day of sacrifice.

2.8.5. Method of Study Performance

2.8.5.1. Fo Generation Female Rats

Rats were observed for viability at least twice each day of the study. These rats were also examined for clinical observations and general appearance at least once. Maternal behavior was recorded daily beginning the day after arrival at the Testing Facility. Body weights were recorded on the day after arrival and before sacrifice (terminal weight). Feed consumption was monitored as feed was replenished on an as-needed basis.

2.8.5.2. F1 Generation Male Rats

Rats were observed for viability at least twice each day of the study. These rats were also examined for clinical observations and general appearance daily (recorded by litter) during the acclimation period and daily during the exposure period. Body weights were recorded once during the acclimation period, daily during the exposure period and at sacrifice (terminal weight). Feed consumption values were recorded twice weekly during the exposure period.

Beginning at the start of week 3 of the exposure period, blood samples (at least 1.6 mL each) were collected bi-weekly (every other week) from each male rat assigned to study. The time of each blood collection was recorded in the raw data. Blood samples were collected at approximately the same time each week of collection (standardized for time of day, between 8:30 am and 11:00 am EDT) to address the circadian, pulsatile release of male hormones. Blood was collected from the orbital sinus. The rats were anesthetized using isoflurane/oxygen before sample collection. The samples were transferred into serum separator tubes and spun in a centrifuge. The resulting serum (0.8 mL) was transferred into polypropylene tubes labeled with the protocol number, rat number, group number, dosage level, day of study, collection interval, date of collection, species, generation and storage conditions. All samples were immediately frozen on dry ice and maintained frozen (approximately -80°C) until analysis at the Testing Facility. Samples were analyzed at the Testing Facility for LH (luteinizing hormone), FSH (follicle-stimulating hormone) and testosterone. All hormones were analyzed using Enzyme Linked Immunosorbent Assay (ELISA) methodology (Amersham Pharmacia Biotech Ltd. - Rat Lutenizing Hormone - Catalog number RPN 2562; Amersham Pharmacia Biotech Ltd. - Rat Follicle Stimulating Hormone - Catalog number RPN 2560 and Biomeda - Testosterone - Catalog number EU 1048).

Subject: 70 Federal Register 23877: Butylparaben
Date: Monday, June 6, 2005 2:28 PM
From: Linda Loretz <loretzl@ctfa.org>
To: <masten@niehs.nih.gov>

Dear Dr. Masten,
Attached are electronic copies of documents submitted by the Cosmetic, Toiletry, and Fragrance Association in response to the Request for Additional Information on Toxicological Study Nominations to the National Toxicology Program (70 Federal Register 23877): Butylparaben. A hard copy has already been sent via Fed Ex.
Best Regards,
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